S71
.E5t

TECHNICAL BULLETIN 51 OCTOBER 1927

University of Minnesota
Agricultural Experiment Station

The Inheritance of Reaction to Ustilago Zeae in Maize

F. R. Immer
Division of Farm Management, Agronomy, and Plant Genetics

UNIVERSITY FARM, ST. PAUL

TECHNICAL BULLETIN 51 OCTOBER 1927

University of Minnesota
Agricultural Experiment Station

The Inheritance of Reaction to Ustilago Zeae in Maize

F. R. Immer
Division of Farm Management, Agronomy, and Plant Genetics

UNIVERSITY FARM, ST. PAUL

CONTENTS

ILLUSTRATIONS

THE INHERITANCE OF REACTION TO *USTILAGO ZEAE* IN MAIZE[1]

F. R. IMMER[2]

Introduction

Ustilago zeae (Beckm.) Unger is one of the most destructive pathogenes affecting corn. The annual losses in the United States from this organism, in 1924, 1925, and 1926 were estimated at 1.8, 1.79, and 1.6 per cent of the total crop (57, 58, 59).[3] During years when the prevalence of corn smut approached a smut epidemic, smut destroyed the ears of from 10 to 15 per cent of some self-fertilized lines of corn grown at University Farm.

Because the organism causing corn smut can live as a parasite on corn plants or as a saprophyte in the soil for a long time, its control by cultural practices is very difficult. Infections on the plants are entirely local, making seed treatment useless. Obviously, the only method by which corn smut can be controlled is the production of resistant varieties of corn.

Corn is largely cross-pollinated (31) and the factors determining its characters are, as a consequence, largely in a heterozygous condition. In order to study the inheritance of such maize characters as resistance to disease, it is necessary first to obtain strains which are homozygous for the factors determining the characters in question. This can be accomplished by producing pure lines by selfing. Once strains have been produced which are pure for the characters in question, crosses can be made between strains differing in reaction, under given environmental conditions, and the mode of inheritance of the characters under investigation can be determined. Inbreeding decreases variability and leads to homozygosity. Many characters normally carried in the recessive condition can be isolated by inbreeding. The only result of inbreeding from an inheritance standpoint is the isolation of pure lines. Abnormal morphological, pathological, or otherwise undesirable characters are isolated in this way and may be eliminated.

The study reported in this bulletin has been a part of the general study of disease resistance conducted by the sections of plant breeding and plant pathology. The problem upon which this discussion

[1] Submitted also to the faculty of the University of Minnesota as a thesis in partial fulfilment of the requirements for the degree of Doctor of Philosophy; granted June 13, 1927.

[2] Grateful acknowledgment is made to Dr. H. K. Hayes, under whose direction these studies were made; to Dr. E. C. Stakman and Dr. J. J. Christensen for help with the pathological phases of the problem; and to Dr. R. J. Garber for assistance in conducting a co-operative experiment reported here.

[3] Reference by number is to "Literature Cited," p. 52.

is based is a continuance of the investigation of the inheritance of reaction to *Ustilago zeae* in maize begun by Hayes, et al (32), and the determination, if possible, of the mode of inheritance of resistance or susceptibility to smut.

REVIEW OF LITERATURE

Piemeisel (56) concluded that healthy, vigorous plants from two to three feet high were most susceptible to infection by the smut-producing organism. Injury tended to increase the chances for infection but was not necessary for successful infection.

Hitchcock and Norton (33), in 1896, noticed that successively the leaves, tassels, ears, and lower nodal buds in turn became principal areas for infection because of the meristematic condition of these parts of the plant. When young leaves were infected many of the infected areas did not develop sufficiently to produce mature spores, owing, probably, to the rapid maturing of the leaf tissue and the consequent inability of the fungus to spread through the older tissue (56).

Potter and Melchers (60) concluded that infection depends not so much on the time of the season as on the stage of development of the host plant. Moisture is probably a factor in the infection of certain parts of the corn plant, but these investigators concluded that it is exceedingly doubtful, particularly in the case of ear and nodal infections, if moisture can be considered in any material degree a limiting factor in smut development under such climatic conditions as are required for the maturity of maize.

Both Pammel and Stewart (55) and Potter and Melchers (60) noted that when smut boils appear on one part of the corn plant there is a strong tendency for infections to appear also on other parts of the plant. The lower nodal buds, or rudimentary pistillate inflorescences, are some of the most common points of infection (60). Pammel and Stewart (55) concluded that if one smut boil makes its appearance on the lower nodes others often make their appearance farther up.

The location of smut boils on the plant has been found by Garber and Quisenberry (25), Hayes et al (32), and Immer and Christensen (36), to be a strain characteristic. Different strains have the majority of smut infections on different parts of the plants. This tendency is inherited from year to year.

Several investigators have studied the inheritance of smut reaction in corn. Jones (37) made crosses of several long-time selfed lines of maize and found that resistance was dominant in the F_1 and that segregation occurred in the F_2. Hayes, et al (32), from six different crosses of selfed lines, concluded that F_1 crosses of resistant strains were more resistant than either parent, while the F_1 crosses between resistant and susceptible strains produced an intermediate degree of infection. Apparently it was easy to isolate self-fertilized lines of corn which differed in their inherited reaction to smut infection in an artificially induced smut epidemic. This indicates that only a few main genetic factors were involved in determining resistance or susceptibility to smut infection.

Garber and Quisenberry (25) isolated selfed lines of corn in which the percentage of infected plants varied from 0.0 to 77.0 per cent. It was not difficult to isolate strains with an appreciable degree of resistance to smut. Resistance was constant in successive seasons' trials.

Immer and Christensen (36) presented further data on some of the selfed lines and crosses first reported by Hayes, et al (32). Their results corroborated the previous Minnesota studies in that dominance of resistance or susceptibility to smut was lacking. Further study was found to be necessary before the number of factors involved in determining certain reactions of selfed lines and crosses to corn smut could be determined.

Coffman, Tisdale, and Brandon (7), from observations of the progeny of ears from the same parent ear rows, in ear-to-row breeding tests, found differences in susceptibility to smut when corn was grown under normal field conditions. These differences were due to different female parents.

Several investigators have attempted to infect corn seedlings grown in the greenhouse, with smut. Sartoris (61) inoculated seedlings grown in sterile test tubes in the greenhouse with mycelium of *Ustilago zeae* obtained from pure cultures, but no infection resulted. He concluded that mycelium obtained in culture is unable as such to infect the host.

Arthur and Stewart (3) germinated corn between moist cloths and sprayed the seedlings with smut conidia when the plumules were about 1.5 inches long. The seedlings were then kept between moist cloths for 24 hours before being transplanted to pots. Seven of the thirteen plants inoculated developed smut.

Tisdale and Johnston (71) found corn seedlings to be as susceptible to smut infection as older plants. The same strain differences found in the field were found to exist in the greenhouse. High temperatures (80° to 95° F.) were found to favor infection. It was concluded that corn seedlings escaped infection under field conditions because of low temperature during the seedling stage. Resistant strains of corn inoculated with smut conidia were infected in the seedling stage, especially when very young, but were able to suppress invasion of the fungus to a certain extent, while susceptible plants were often killed. Resistance was internal and physiological rather than morphological. It was concluded that some internal reaction on the part of the resistant plant holds the fungus in check within the plant. Some results obtained by the use of smut from different states suggests that different physiologic forms of smut may have been used.

The work of several investigators foreshadowed the actual demonstration of the existence of physiologic forms of *Ustilago zeae*. Melchers (54), in 1921, found that cultures of smut seemed to differ in their ability to produce infections when plants were artificially inoculated. Selections and crosses of corn resistant to smut infection in one state were often more susceptible in another state. Tisdale and Johnston (71) found a difference in the ability of smut from different sources to infect corn seedlings. It remained, however, for Stakman and Christensen (68) to suggest the existence of several physiologic forms of corn smut and for Christensen and Stakman (6) actually to demonstrate the existence of at least twelve physiologic forms of *Ustilago zeae* on culture media and of seven physiologic forms differing parasitically. Some forms mutated very rapidly on culture media while others did not (6). It seems logical to conclude that a large number of physiologic forms of corn smut exist under normal field conditions.

The inheritance of quantitative characters in plants has been studied by several investigators by means of a study of their linkage relations with factors located in known linkage groups. Lindstrom (49, 52) has presented very good evidence that size factors in the tomato are linked in inheritance with the two qualitative color factors Rr and Yy.

Quantitative inheritance in the garden bean has been studied by two investigators. Sirks (65) advanced the hypothesis that size was dependent on factors controlled by an inhibiting factor, Ii, linked with a recessive color factor. Sax (62, 63, 64) presented data on linkages between seed weight and mottling and pigmentation of the seedcoat, seed weight and vine type, and yield and seedcoat characters. Sax concluded that the inheritance of yield can be determined only by a study of the linkage relations between qualitative factors whose linkage relations are known, and the factors determining yield.

Griffee (26, 27) studied the inheritance of resistance to *Helminthosporium sativum* Pammel, King and Baake in relation to certain qualitative characters in a cross of two varieties of barley. Resistance was found to be associated in inheritance with the independently inherited factor pairs for 2-rowed vs. 6-rowed, rough vs. smooth awns, and white vs. black glumes.

The inheritance of such a quantitative character as smut reaction in corn will, in all probability, be most easily determined by establishing the linkage relations between the factors determining smut reaction and known factors in the chromosome linkage groups.

EXPERIMENTAL METHODS AND MATERIALS

The selfed lines used as parents in making the crosses for this inheritance study were produced under "smut epidemic conditions" in a co-operative investigation carried on by the sections of plant breeding and plant pathology. The reactions of the parent lines from 1920 to 1924, inclusive, and of the F_1, F_2, and backcrosses between these parent lines have been previously reported (32, 36). The parent lines for these crosses were selfed lines of Minnesota No. 13, Rustler, and Longfellow. The first two varieties mentioned are dents while the last is a flint variety. The crosses were confined to selfed lines within a variety. The parental strains had relatively the same percentage of smutted plants for several years and were consequently assumed to be practically homozygous for a certain manner of reaction under conditions favorable for infection by the pathogene.

The corn was grown in rows of 28 or 42 hills each, the hills being spaced one foot apart in the row. Two seeds were planted in each hill, when sufficient seed was available, and the hills were thinned to a single plant basis when the plants were about six inches high. Except in a few cases when sufficient seed was not available, each strain or cross was replicated in two systematically distributed rows. The F_2 linkage study material, however, was grown in a separate plot in 1926, only one seed was planted per hill and the cultures were not replicated.

All plants in each culture were given a number, and notes were taken on the location and the amount of infection, if any, on each plant at three different times during the summer. The first notes were taken about August 1 for the purpose of noting the early plant infections, particularly on the leaves. About August 25 notes were again taken; this time stalk infections predominating. Notes were taken a third time about September 20, primarily to obtain data on ear infections, which were not evident on August 25. In 1926 notes were taken only twice because of abnormal weather conditions—on August 16 and September 15. In the F_2 linkage study, data were taken but once and then when it was thought that maximum infection of the plants had been reached.

The location of smut infections on the plants was noted as: S, sucker; M, main stalk; L, leaf; Se, leaf sheath; T, tassel; Mb, main stalk base; and Sh, shoot or rudimentary ear bud. Multiple infections were recorded as Mi, and incipient infections as In. The smut boils were classified into five groups according to size: In, incipient; W, warty; S, smaller than one inch in one or two diameters; M, larger than S and smaller than L; and L, larger than 4 inches in more than one diameter (see Figs. 1 and 2). In summing up the notes on total infection, the percentage of plants infected, barring incipient infection, was taken as the total percentage of infection for the strain. The smut boils classified as "incipient" dried up before ripening.

A smut epidemic was induced artificially by two methods: (1) smut spores were mixed with manure and this was spread over the plot when the seedlings were from 3 to 6 inches high. (2) Beginning when the plants were about 2 feet high and continuing until they were almost mature, the plants were sprayed with a water suspension of smut sporidia, in the evening, about every ten days. This was done under the direction of J. J. Christensen, of the section of plant pathology.

Selfed lines of corn which were apparently highly resistant to smut in the plant breeding nursery, as determined from several years' notes, were grown in the "smut inheritance plot" to determine to what extent it was possible to isolate smut-resistant strains of corn under non-epidemic conditions, when the corn was grown under a systematic rotation, as compared to the reaction of these strains when grown in an artificially induced smut epidemic.

Thirty-four selfed lines which were being used as parent lines for single and double crosses in the plant breeding nursery, were grown in the smut inheritance plot in 1926 in order more accurately to test the manner of their reaction to smut. These strains were not selected because they were thought to be resistant to smut infection but because of their general desirability as parent lines for making crosses, as judged from several years' observations and notes, one of which was the note on smut infection.

Eight low smut strains, proved to be such under epidemic conditions, were combined in 1925 in order to determine whether the same genetic factors were concerned in determining resistance in all parent lines of such a combination. In making the combination, pollen was collected from seven of the eight strains, mixed, and used to pollinate the eighth strain, and so on until all the parent strains had been pollinated by a mixture of pollen of the other seven strains. The progeny of each female parent was grown separately in 1926.

During 1925 and 1926 a co-operative experiment was carried on by the sections of plant breeding and plant pathology at the University of Minnesota and the department of agronomy of the University of West Virginia. Ten selfed lines of corn from each experiment station, of known reaction in a smut epidemic, were exchanged and these twenty strains were grown at both experiment stations in 1925 and 1926. It was hoped that this would give an index to the reaction of different selfed lines under different environmental conditions and against, possibly, different physiologic forms of corn smut.

Selfed lines of corn with known genetic factors in one or more of the eight established chromosome linkage groups were also grown in the smut inheritance plot, for the purpose of determining their resistance or susceptibility to smut in order that they might be crossed with other selfed lines differing in smut reaction and one or more known genetic factors; and the presence or absence of linkage between factors determining smut reaction and these qualitative factors determined. It was hoped that the location of the factors determining resistance or susceptibility to smut might be established in one or more of the chromosome linkage groups and their inheritance more definitely determined. Crosses were made, therefore, between strains differing in these known genetic factors and in smut reaction. Six such crosses, involving nine different factor pairs, located in six different chromosome linkage groups, were made in 1924; and four more crosses, involving four more factor pairs and adding one more linkage group, were made in 1925. The F_1 crosses were grown in 1925 and 1926. The F_2 generation of the 1924 crosses was grown in 1926.

Seedling infection in the greenhouse was studied by hypodermically inoculating F_3 lines of seedlings grown from seed of forty-one F_2 plants selected at random from a cross of Rustler high × low smut lines. The same F_3 lines had been grown in the field in both 1925 and 1926. Seed of the same F_2 ears was used both years in the field as well as for the greenhouse test. A preliminary greenhouse test of the susceptibility of the seedlings from the parent lines gave inconclusive results, owing to the small number of plants involved. An insufficient amount of seed prevented the parent lines from being tested a second time.

The seeds were planted in sand in the greenhouse bench on December 16, 1926, in rows crosswise of the bench, sixteen seeds being planted per row and each culture replicated twice, that is, grown in three systematically distributed rows. The seeds were spaced 2 inches apart in rows 3 inches apart. On January 8, the seedlings were artificially inoculated with sporidia in carrot decoction, from a single physiologic form of *Ustilago zeae* obtained from Dr. Christensen. This physiologic form was originally isolated from a smut infection on a plant in a Longfellow low smut culture grown at University Farm.

The seedlings were inoculated by hypodermic injection of smut conidia into the plants in two places—the growing point of the plant and again about an inch higher. Notes were taken on both the place and the degree of infection of the individual seedlings, on one replicate on January 19 and on the other two replicates on January 21. In calculating the percentage of seedlings infected by smut in a given F_3 line, only those plants were considered as smutted which had developed definite smut boils (See Fig. 7). Plants with only small pimples on the leaves or chlorotic areas surrounding the point of inoculation of the leaves were not considered smutted.

EXPERIMENTAL RESULTS

In order to study the inheritance of resistance to smut, it was first necessary to isolate parent strains which were apparently homozygous for the factors determining a particular type of smut reaction. This homozygosity was reached by the method of selection in self-fertilized lines. Considering the apparent ease with which Hayes, et al (32), and Garber and Quisenberry (25) succeeded in isolating selfed lines of corn breeding true for a particular type of smut reaction, it appears that only a few main factors were involved in determining resistance. The manner of inheritance of these factors was unknown, however.

Each of the selfed strains and crosses in the smut inheritance plot was grown in two systematically distributed plots. The correlations between the percentage of total plants infected in the first and second series of these plots in 1924, 1925, and 1926 are given in Tables I, II, and III.

TABLE I

CORRELATION BETWEEN PERCENTAGE OF TOTAL SMUT INFECTION IN FIRST AND SECOND SERIES OF A UNIFORM REPLICATION IN 1924

Percentage of total smut in second series	Percentage total smut in first series: 3	10	17	24	31	38	45	52	59	66	73	80	87	94	Total
3	2	1	1												4
10	3	3	2	1					1						10
17	3	4		1	1	1									10
24			3	5	1										9
31		1	4	1	1		1					1			9
38	1			2	2	3	2			1					11
45						3	2	1	1						7
52	1						1	1							3
59						3	1	2							6
66							1		1						2
73									1	1	1	1			4
80										1	1				2
87											1	1			2
94									1					1	2
Total	10	9	10	10	5	10	8	4	5	3	3	3		1	81

$r = +0.7982 \pm 0.0272$

TABLE II

CORRELATION BETWEEN PERCENTAGE OF TOTAL SMUT INFECTION IN FIRST AND SECOND SERIES OF A UNIFORM REPLICATION IN 1925

Percentage total smut in second series \ Percentage total smut in first series	2.5	8.5	14.5	20.5	26.5	32.5	38.5	44.5	50.5	56.5	62.5	68.5	74.5	80.5	86.5	92.5	98.5	Total
2.5	3		1			1												5
8.5		5				1												6
14.5	1	1		2	1	1	1											7
20.5		1	3	2	1	3	2				1							13
26.5			1			1	1		1	1	1							6
32.5		1		1	1		1	1			1							6
38.5				2			4					1	2					9
44.5				1		1	2	1	2		2							9
50.5				1		1	3	4			3	1		1		1		15
56.5					3	1	1	1	5	3		2						16
62.5					2		1		2	1		4	2		2			14
68.5							1		1	1	1	1	4		1	1		11
74.5											1	2	1		1	2		7
80.5							1			1	1		3	2	1	3		12
86.5											2	1		1	3	2	3	12
92.5														2	1		1	4
98.5												1			1	5	6	13
Total	4	8	5	9	8	10	18	7	11	7	13	13	12	6	10	14	10	165

$$r = +0.8316 \pm 0.0162$$

TABLE III

CORRELATION BETWEEN PERCENTAGE OF TOTAL SMUT INFECTION IN FIRST AND SECOND SERIES OF A UNIFORM REPLICATION IN 1926

Percentage total smut in second series \ Percentage total smut in first series	2.5	8.5	14.5	20.5	26.5	32.5	38.5	44.5	50.5	56.5	62.5	68.5	74.5	80.5	86.5	92.5	98.5	Total
2.5	5	4	1															10
8.5	4	4	3	1	1													13
14.5	3	4	4	5	2	3	1		1									23
20.5	1	3	2	2		2	2											12
26.5		1	2	2	1			2	1									9
32.5			3	1	3		2	1		1								11
38.5		1	5		3	3	2	2			1							17
44.5				1	1	1		1	2		1							7
50.5		1	1		1	4	1	1	1	2								12
56.5				2		1	2	1	1	1	1	1		2		1		12
62.5			2	1	1	1	1	3										10
68.5							1	2	1	1	1	1		1				8
74.5									1			2		1				4
80.5							1			3	1	1	1	2				9
86.5							1					1				2		4
92.5										1					1			2
98.5														1			1	2
Total	13	18	23	15	13	15	14	13	8	9	5	6	1	7	1	3	1	165

$$r = +0.7750 \pm 0.0210$$

The correlation coefficients were high, as would be expected, indicating that the strains reacted in very nearly the same way when grown in different parts of the field.

The probable errors were calculated by the pairing method first suggested by Wood and Stratton (73).[4] This formula gives the probable error in percentage for the mean of the two plots of each strain systematically replicated.

The probable error for the average of several years was computed from the formula $\frac{1}{N}\sqrt{a^2+b^2\ldots\ldots n^2}$ where N = the number of seasons in which the probable errors were obtained and a + b . . . . n represents the separate errors for each year.

The probable error in percentage of the mean of two plots systematically replicated, as calculated by the pairing method, was determined for the groups of strains with a percentage of infection between two arbitrary limits. The probable errors of each such class or group of selfed lines, in 1924, 1925, and 1926, and the number of pairs of strains in each class from which the probable error was calculated, are given in Table IV.

TABLE IV

PROBABLE ERROR IN PERCENTAGE OF SMUT INFECTION IN SELFED LINES AND CROSSES DIFFERING IN PERCENTAGE OF SMUT INFECTION AS CALCULATED BY THE PAIRING METHOD

Percentage total smut in class	1924		1925		1926	
	Pairs in class	P.E. in per cent	Pairs in class	P.E. in per cent	Pairs in class	P.E. in per cent
0- 10	7	53.7	10	17.4	20	41.2
10- 20	14	26.9	8	20.4	28	17.9
20- 30	19	14.4	18	19.8	25	20.9
30- 40	9	14.0	11	10.0	28	19.8
40- 50	12	7.5	24	14.8	19	12.4
50- 60	9	10.1	26	9.9	16	11.0
60- 70	11	5.2	15	6.9	10	10.7
70- 80			19	5.4	13	7.8
80- 90	..	...	15	4.2	6	2.7
90-100	..	...	19	2.5		
0-100	81	17.1	165	10.5	165	18.4

The probable error is seen to decrease rapidly as the percentage of smut infection increases. The reason for this is readily observed. While the actual deviations from the means in the low smut strains is smaller than in the high smut strains, the percentage of deviation is far higher for the low than for the high smut strains. A high probable error for the low smut strains is to be expected because a deviation,

[4] $\text{P.E.} = \frac{\frac{\Sigma\ (\text{dev. in } \%)}{n}}{\sqrt{N}}$ where Σ = summation, dev. in % = deviations in percentage of infection of each plot of a selfed strain or cross from the mean of both plots of that strain, n = the number of deviations in percentage, and N = the number of plots in each strain.

even tho small, gives a high percentage of error. The probable errors for the entire experiment were 17.1, 10.5, and 18.4 per cent for the years 1924, 1925, and 1926. It is obviously unfair to use these average probable errors for all strains regardless of their percentage of smut infection, because such probable errors would be too lenient with the low smut strains and would have an opposite effect on the high smut strains. The smaller number of low smut strains in the 1925 test explains the low (10.5 per cent) average probable error for that year. The probable errors of the strains and crosses in the 1924, 1925, and 1926 tests were, therefore, calculated from the probable errors in per cent in the classes into which the strains fell, as given in Table IV. The probable errors for past years, when used, were those previously reported by Hayes, et al (32).

Reactions of Parent Strains and Crosses to Smut Infection

Eleven selfed lines of corn which seemed to be homozygous for a particular reaction to *Ustilago zeae* in a smut epidemic, were selected as parent strains to be used in making crosses for a study of the inheritance of smut reaction. The percentage of total and of ear smut infection on these parent lines from 1921 to 1926, inclusive, is given in Table V. Ear smut, being very important from an economic standpoint, was calculated separately.

TABLE V

PERCENTAGE OF TOTAL AND EAR SMUT INFECTION OF SELFED LINES OF CORN WHICH WERE USED AS PARENT LINES IN MAKING CROSSES

Variety	Cult. No.	Percentage of total smut						
		1921	1922	1923	1924	1925	1926	Average
Rustler	28	84.5	100.0	98.0	83.8	100.0	89.2	92.6±3.6
do	38	28.5	2.5	12.1	33.4	42.3	41.7	26.8±1.7
do	33	2.0	2.2	12.6	4.3	3.5	0.0	4.1±0.5
Minn. No. 13	30	58.0	62.8	85.5	74.4	97.4	52.2	71.7±2.9
do	31	14.0	40.0	21.8	29.4	47.7	48.2	33.5±2.0
do*	17	10.5	65.6	14.0	...	...	...	....
Longfellow	41	73.0	83.7	65.9	...	95.7	79.5	79.6±3.4
do	40	12.0	27.7	69.1	52.4	77.7	69.0	51.3±2.5
do	42	65.0	84.0	67.1	27.3	27.1	17.4	48.0±2.9
do	43	4.5	19.4	7.4	15.0	8.8	9.5	10.8±1.1
King Phillip	39	58.0	84.0	97.5	...	100.0	78.0	83.8±4.0
		Percentage of ear smut						
Rustler	28	11.2	21.3	28.0	5.6	26.0	15.2	17.9
do	38	14.4	0.0	4.0	8.0	7.7	18.1	8.7
do	33	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Minn. No. 13	30	32.0	26.6	16.8	36.5	26.2	10.9	24.8
do	31	0.0	4.0	4.5	0.0	3.1	7.4	3.2
do*	17	0.0	0.0	2.0	...	...	...	...
Longfellow	41	35.4	20.9	26.5	...	19.5	8.8	22.3
do	40	4.8	9.7	13.0	47.6	44.5	50.0	28.2
do	42	12.4	6.0	30.6	5.5	16.7	4.3	12.6
do	43	0.0	2.5	0.0	0.0	1.8	0.0	0.7
King Phillip	39	41.7	51.3	95.0	...	100.0	63.4	70.3

* Culture 17 was lost in 1923.

Eight of the strains gave nearly uniform results from year to year, indicating that the factors determining the particular type of smut reaction were in a practically homozygous condition. Cultures 17, 40, and 42 showed considerable variability.

Culture 17 was much more susceptible to smut in 1922 than in 1921 or 1923, 44.7 per cent of the infections in 1922 being due to tassel infection alone. Tassel infections were low in this strain in the other two years. Culture 42 had an average of 72.0 per cent of the plants infected by smut during the first three years of the test and only 23.9 per cent during the last three years. Apparently, the genotypic condition which determined the reaction of this strain from 1921 to 1923, inclusive, was not the same as that which determined its reaction from 1924 to 1926. The reverse was true with ear smut in culture 40. In this strain an average of 9.2 per cent of the plants were infected by ear smut during the period 1921 to 1923, and 47.3 per cent from 1924 to 1926. Culture 40 had a much higher percentage of smutted plants during the last four years of the test than during the first two years.

The relationship between total smut infection and amount of ear smut was not always constant, even within a variety. Cultures 40 and 42, in the variety Longfellow, had very nearly the same percentage of total smut for an average of six years, but twice as much ear smut was produced on culture 40 as on culture 42 during this period. The King Phillip culture, No. 39, had an extremely high percentage of ear smut. Since smut in corn is a pathological condition dependent on environmental conditions for dissemination of the spores and consequent chance for infection, some variability of infection is to be expected from year to year.

These parent lines were classified as high, medium, and low smut strains according to the average percentage of infection from 1921 to 1924, inclusive. Thus, the strains with an average of 0.0 to 15.0 per cent of total smut were classified as low smut strains; those with an average between 15.0 and 52.0 per cent were called medium smut strains; and those with an average of over 52.0 per cent infection were called high smut strains. Figure 3 shows the high smut strain of Rustler which was used in these crosses. While varying somewhat, the parent lines (except cultures 17, 40, and 42) were quite uniformly within these class limits from year to year. Culture 42 was a high smut line in the first three years of the test and a medium smut line in the last three years. In culture 40 there was a steady increase in ear smut infection from 1921 to 1926.

The F_1 crosses were grown in 1923 and again in 1924, when sufficient seed was available; 1922 seed was used both years. A summary of the percentage of total and ear smut infection in these F_1 crosses is given in Table VI.

TABLE VI

PERCENTAGE OF TOTAL AND EAR SMUT INFECTION IN F_1 CROSSES

Variety	Cult. No.	Type of smut reaction	Percentage total smut			Percentage ear smut		
			1923	1924	Av.	1923	1924	Av.
Rustler	28×33	High×Low	25.0	...	....	4.5	...	...
do	38×33	Med.×Low	3.0	2.6	2.8±0.8	0.0	0.0	0.0
Minn. No. 13 ...	30×17	High×Med.	45.1	46.5	45.8±3.9	12.5	22.1	17.3
do ...	17×31	Med.×Med.	8.6	32.5	20.6±2.2	0.0	6.5	3.3
Longfellow	43×42	Low×Med.	60.7	61.2	61.0±4.9	15.9	18.1	17.0
do	43×40	do	24.7	...	....	6.5	...	...

The percentage of total smut infection of the F_1 crosses was nearly the same for both years in three of the four comparable crosses; the percentages in 1923 and 1924 for the cross of lines 17 × 31 varied considerably. The difference between the results of the two years for this cross was five times its probable error. Such a large difference would be expected but once in 1350 trials on the basis of random sampling. In this cross, however, the 1923 data were obtained from only 35 plants grown in two systematically distributed rows, while the 1924 data were obtained from 77 plants grown in a single row.

The F_2 progenies were grown from seed of the selfed ears of the 1923 F_1 plants. The backcrosses of the F_1 with the parent strains were made in 1923 and the progenies were grown in 1924. In eight of the backcrosses it was possible to compare the percentage of smut infection of the progeny from crosses in which the F_1 was used as the male and as the female parent. That is, the F_1 was used as the staminate and as the pistillate parent in crossing back to either or both of the parent strains. A comparison of the percentage of smut found when the crosses were compared with their reciprocals is given in Table VII.

TABLE VII

COMPARISON OF PERCENTAGE OF TOTAL SMUT IN COMPARABLE CROSSES WHEN THE F_1 WAS USED AS THE STAMINATE AND WHEN USED AS THE PISTILLATE PARENT

Cross	Percentage total smut		F_1 as ♂ minus F_1 as ♀
	F_1 as ♂	F_1 as ♀	
Med. × High × High	74.0±3.9	74.3±3.9	− 0.3±5.5
Low × Med. × Med.	72.8±3.8	53.0±5.4	+19.8±6.6
Med. × Med. × High	55.0±5.6	45.2±3.4	+ 9.8±6.6
Med. × Med. × Med.	32.9±4.6	30.0±4.2	+ 2.9±6.2
do	22.8±3.3	22.5±3.2	+ 0.3±4.6
Low × Low × High	18.7±5.0	26.2±3.8	− 7.5±6.3
Low × Med. × Med.	9.6±5.2	10.8±2.9	− 1.2±6.0
Low × Low × Med.	9.5±5.1	6.1±3.3	+ 3.4±6.1

In only one of the eight crosses compared with its reciprocal was the difference as high as one and one-half times its probable error. In this one cross the difference was three times its probable error, which means that the odds were 22.6 to 1 that a deviation as great as the designated one or greater would be expected on the basis of random sampling. It may be concluded from these data that the factors for smut resistance or susceptibility were transmitted in the same manner in both male and female gametes.

In Table VIII is summarized the percentage of total and of ear smut infection in the parent lines and in the F_1, F_2, and backcrosses of these lines. The percentage of the parent lines and of the F_1 crosses is the average infection of these strains as given in Tables V and VI.

TABLE VIII

SUMMARY OF REACTIONS OF PARENT LINES, AND OF THE F_1 F_2, AND BACKCROSSES OF THESE LINES, TO ATTACKS OF *Ustilago zeae*

Variety	Parent line or cross	Type of smut reaction	Generation selfed or cross	Per cent total smut	Per cent ear smut
Minn. No. 13 ...	17	Medium	4	30.0±4.2	0.7
do ...	31	do	7	33.5±2.0	3.2
do ...	17 × 31	Med. × Med.	F_1	20.6±2.2	3.3
do ...	17 × 31	do	F_2	26.2±3.8	7.7
do ...	31 × (17 × 31)	Med. × (Med. × Med.)	Parent × F_1	32.9±4.6	5.0
do ...	17 × (17 × 31)	do	Parent × F_1	22.8±3.3	6.2
do ...	(17 × 31) × 31	(Med. × Med.) × Med.	F_1 × Parent	30.0±4.2	3.8
do ...	(17 × 31) × 17	do	F_1 × Parent	22.5±3.2	3.2
do ...	30	High	7	71.7±2.9	24.8
do ...	17	Medium	4	30.0±4.2	0.7
do ...	30 × 17	High × Med.	F_1	45.8±3.9	17.3
do ...	30 × 17	do	F_2	52.2±5.3	22.1
do ...	17 × (30 × 17)	Med. × (High × Med.)	Parent × F_1	55.0±5.6	14.6
do ...	30 × (30 × 17)	High × (High × Med.)	Parent × F_1	74.0±3.9	22.2
do ...	(30 × 17) × 17	(High × Med.) × Med.	F_1 × Parent	45.2±3.4	9.3
do ...	(30 × 17) × 30	(High × Med.) × High	F_1 × Parent	74.3±3.9	23.6
Rustler	28	High	7	92.6±3.6	17.9
do	33	Low	7	4.1±0.5	0.0
do	28 × 33	High × Low	F_1	25.0±3.6	4.5
do	28 × 33	do	F_2	31.4±4.8	11.1
do	33 × (28 × 33)	Low × (High × Low)	Parent × F_1	18.7±5.0	6.3
do	(28 × 33) × 33	(High × Low) × Low	F_1 × Parent	26.2±3.8	5.7
do	38	Medium	7	26.8±1.7	8.7
do	33	Low	7	4.1±0.5	0.0
do	38 × 33	Med. × Low	F_1	2.8±0.8	0.0
do	38 × 33	do	F_2	12.9±3.5	2.4
do	38 × (38 × 33)	Med. × (Med. × Low)	Parent × F_1	9.6±5.2	1.9
do	33 × (38 × 33)	Low × (Med. × Low)	Parent × F_1	9.5±5.1	2.4
do	(38 × 33) × 38	(Med. × Low) × Med.	F_1 × Parent	10.8±2.9	0.0
do	(38 × 33) × 33	(Med. × Low) × Low	F_1 × Parent	6.1±3.3	1.2
Longfellow	43	Low	7	10.8±1.1	0.7
do	42	Medium	7	48.0±2.9	12.6
do	43 × 42	Low × Med.	F_1	61.0±4.9	17.0
do	43 × 42	do	F_2	47.5±3.5	12.0
do	42 × (43 × 42)	Med. × (Low × Med.)	Parent × F_1	72.8±3.8	22.8
do	(43 × 42) × 43	(Low × Med.) × Low	F_1 × Parent	22.4±3.2	6.5
do	(43 × 42) × 42	(Low × Med.) × Med.	F_1 × Parent	53.0±5.4	14.3
do	43	Low	7	10.8±1.1	0.7
do	40	Medium	7	51.3±2.5	28.2
do	43 × 40	Low × Med.	F_1	24.7±3.5	6.5
do	43 × 40	do	F_2	16.8±4.5	10.5
do	(43 × 40) × 43	(Low × Med.) × Low	F_1 × Parent	28.6±4.1	5.8
do	(43 × 40) × 40	(Low × Med.) × Med.	F_1 × Parent	31.2±4.4	31.5

In the Minnesota No. 13 medium $\times$ medium smut cross, the F_1 was slightly more resistant than the more resistant parent. If, however, the high percentage of tassel smut in culture 17 in 1923 (an abnormal condition) is eliminated, the F_1 was intermediate in reaction between the parents. The F_2 was slightly more susceptible than the F_1. In the backcross with culture 17 the percentages of total smut were lower than when backcrossed with culture 31. Culture 17 was the more resistant of the two parent lines of this cross.

In four of the other crosses the F_1 and F_2 gave an intermediate reaction between the parents. In the Longfellow low $\times$ medium smut cross, the medium smut parent was much more susceptible to smut infection the first three years of the test (1921-23, inclusive) than it was the last three years (1924-26, inclusive). The F_1 crosses were made in 1922 and the F_2 and backcrosses in 1923. The genotypic constitution of this parent strain was probably different when these crosses were made from that during the last three years of the test. This might account for the fact that the percentage of infected plants in the F_1 was higher than in the six-year average of either parent, and the F_2 reaction was as high as in the more susceptible parent.

The smut reaction in F_2 was very nearly the same as in F_1. In four of the six crosses the difference between the F_1 and the F_2 was less than twice its probable error and in all crosses the difference was less than three times its probable error.

In none of the backcrosses was the percentage of smut infection obtained significantly larger or smaller than that of either of the parents. In each of the nineteen backcrosses the reactions were intermediate, in twelve of the crosses being closest to that of the higher smut parent and in the other seven crosses most closely approaching that of the lower smut parent. In general, it can be concluded from these data that the smut reaction of the crosses was intermediate between the reactions of the parents.

In studying the inheritance of ear smut infection, it was found that in three of the six crosses the F_1 generation was more severely infected than the average of the parents; and in the other three crosses the F_1 was less severely infected than the average of the parents. In the F_2 generation seven of the eight crosses were more susceptible to ear smut infection than was the F_1. Of the nineteen backcrosses, ten were more severely infected with ear smut than either parent and the other nine gave an intermediate reaction, five of these being closest to the higher parent, one being the same as the average of the parents, and the other three crosses being closest to the lower parent.

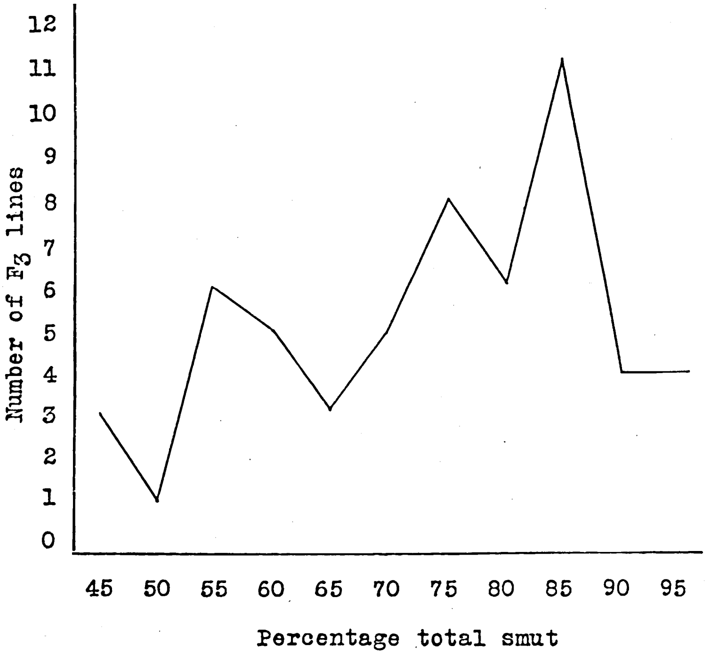
Corn smut, being due to a pathogene which is dependent on environmental conditions for dissemination of the inoculum and consequent chance for infection, and the percentage of infection consequently being quite variable, the inheritance of reaction of strains of corn to this organism can be best studied by self-pollinating a large number of F_2 plants selected at random and studying their nature by growing seed from them in the F_3.

In Table IX is graphically presented a summary of the reactions of 56 F_3 lines of the Minnesota No. 13 high × medium smut cross (30 × 17) grown in 1925. Unfortunately the medium smut parent was lost in 1923 and its reaction could not be compared with that of the F_3 lines grown in 1925.

TABLE IX

SUMMARY OF PERCENTAGE TOTAL SMUT IN F_3 LINES OF A MINN. NO. 13 HIGH × MEDIUM SMUT CROSS (30 × 17), GROWN IN 1925

The high parent had an average of 71.7 per cent smutted plants for an average of six years (97.4 in 1925) and the low parent had an average of 30.0 per cent smutted plants for an average of three years.



The range of variability of these F_3 lines exceeded the six-year average for the high smut parent but did not exceed the smut reaction of the high smut parent in 1925, the year the F_3 lines were grown. More of the F_3 lines resembled the more susceptible parent than the more resistant parent line, fifty-two being high smut lines while only four were medium smut lines in 1925, a very favorable year for a high degree of smut infection. The number of genetic factors involved can not be concluded from these data, however.

F_3 lines of another cross, that of Rustler high × low smut strains (28 × 33) were grown in both 1925 and 1926 from seed of the same F_2 plants. In Table X is shown the correlation between these F_3 lines when grown the two different years.

TABLE X

CORRELATION BETWEEN PERCENTAGE OF TOTAL SMUT INFECTION OF F_3 HIGH × LOW SMUT LINES GROWN IN 1925 AND 1926 FROM SEED OF THE SAME 1924 EARS

Percentage total smut in 1925	Percentage total smut in 1926: 15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	Total
15		2	1	1													4
20		2	3				1										6
25		1	1			1				1							4
30		3			1												4
35	1				2	1	1										5
40				3	1	1	1										6
45		1					1	1	1								4
50			1		2		1	2								1	7
55						1				1		1					3
60	1								1	1							3
65					1	1											2
70									1				1				2
75							1										1
80									1								1
Total	2	9	6	4	7	5	6	3	4	3		1	1			1	52

$r = +0.5574 \pm 0.0654$

The magnitude of the correlation indicates the tendency for the F_3 lines to produce relatively the same percentage of total smut infection when grown in these two years, indicating that genetic factors determined the reaction to smut infection under the given environmental conditions.

In Table XI are graphically summarized the averages of the reactions of these F_3 lines for the two years they were grown.

The variability of the F_3 lines did not extend to the limits set by either of the parent strains. Forty-two of the F_3 lines were medium smut lines while the other ten were high smut lines, as an average of the two years they were tested. No low smut lines were found in the F_3 generation. As in Table IX, more of the F_3 lines resembled the susceptible than the resistant parent line. It would be difficult to conclude from the table how many factors were involved in determining resistance or susceptibility to smut infection. The nature of the distribution of these F_3 lines indicates that several factors were involved.

TABLE XI

SUMMARY OF AVERAGE PERCENTAGE TOTAL SMUT IN F_3 LINES OF A RUSTLER HIGH × LOW SMUT CROSS (28 × 33), GROWN IN 1925 AND 1926

The high smut parent had 94.6 and the low smut parent 1.8 per cent of smut infected plants during the same two years.

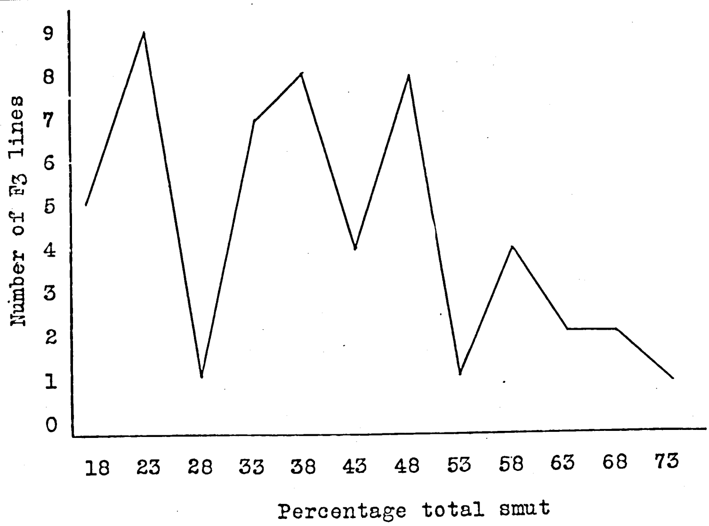
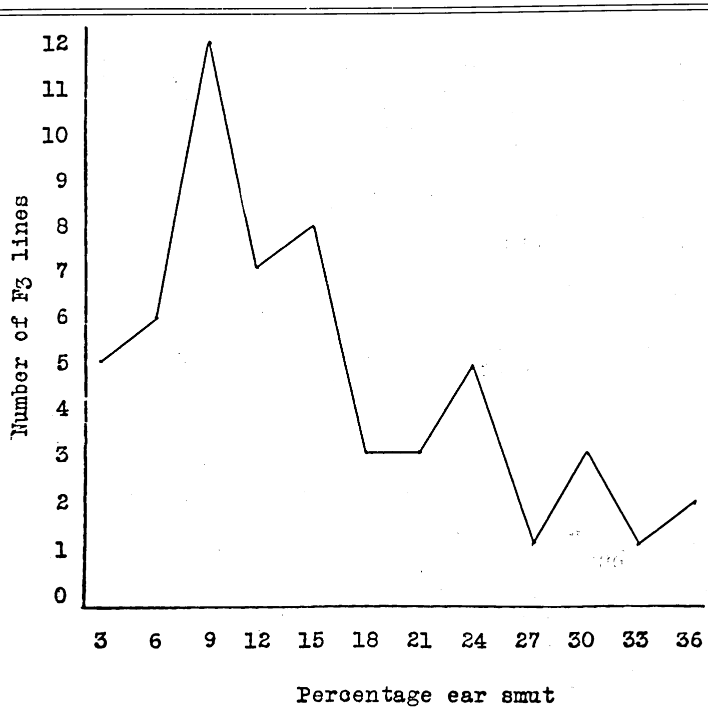


TABLE XII

SUMMARY OF PERCENTAGE EAR SMUT IN F_3 LINES OF A MINNESOTA NO. 13 HIGH × MEDIUM SMUT CROSS (30 × 17), GROWN IN 1925

The high parent had 24.8 per cent smutted ears for an average of six years (26.2 per cent in 1925), and the low parent had 0.7 per cent smutted ears for an average of three years.



The inheritance of reaction to ear smut infection was studied independently of total smut infection. The reaction of the F_3 lines of the Minnesota No. 13 high × medium smut cross (30 × 17) with regard to ear smut infection is shown in Table XII.
In this cross the distribution of the F_3 lines somewhat exceeded the limit set by the high smut parent. Thirty of the F_3 lines had less and twenty-six had more ear smut than the average of the parent strains.

TABLE XIII

Correlation Between Percentage of Ear Smut Infection of F_3 High × Low Smut Lines (28 × 33) Grown in 1925 and 1925 from Seed of the Same 1924 Ears

Percentage ear smut in 1926

Percentage ear smut in 1925	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	21.5	22.5	23.5	24.5	Total
0.5	1		1		1		2			1																6
1.5	2		1					1	1																	5
2.5			1		1	1	1			1																5
3.5	2							1																		3
4.5				1			2	1								1	1									6
5.5					2																					2
6.5	2				1		1	1																		5
7.5	1				1																	1				3
8.5									1																	1
9.5	1	1		1																						3
10.5		1					1																			2
11.5				1						1																2
12.5					1																					1
13.5													1													1
14.5										1							1				1					3
15.5																										
16.5																										
17.5																										
18.5																										
19.5																										
20.5											1															1
21.5										1																1
22.5			1																							1
23.5																										
24.5																										
25.5																										
26.5																										
27.5																										
28.5																										
29.5																										
30.5																										
31.5																										
32.5																									1	1
Total	9	2	4	3	7	1	7	4	2	5	1		1			1	2			1	1				1	52

$r = +0.6311 \pm 0.0563$

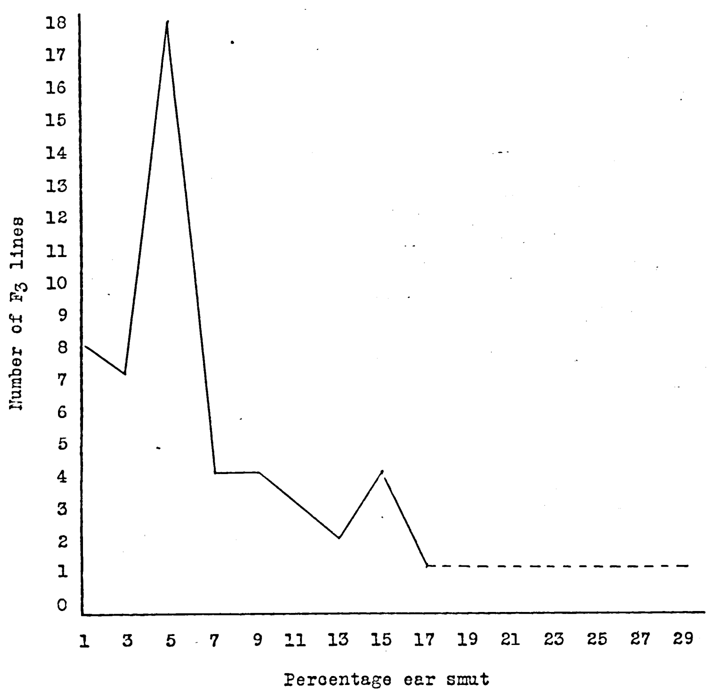
In Table XIII is shown the correlation between the percentage of ear smut infection of the different F_3 lines from the Rustler high × low smut cross (28 × 33) when grown from seed of the same F_2 ears in 1925 and 1926. The correlation coefficient of $+0.6311 \pm 0.0563$ indicates that genetic factors were here also involved in determining the reaction of these F_3 lines to ear smut infection in 1925 and 1926.

In Table XIV is shown the distribution of these F_3 lines with regard to the average percentage of ear smut infection for 1925 and 1926. In this cross, with the exception of a single F_3 line (29 per cent), none of the F_3 lines exceeded the limits of the parents. Forty-one of the F_3 lines had less and eleven had more ear smut than an average of the parent strains for the same two years.

TABLE XIV

SUMMARY OF AVERAGE PERCENTAGE EAR SMUT IN F_3 LINES OF A RUSTLER HIGH × LOW SMUT CROSS (28 × 33), GROWN IN 1925 AND 1926

The high parent had 20.6 and the low parent 0.0 per cent of ear smut for the same two years.



In general, the segregation in the F_3 lines with regard to total smut infection showed a higher percentage of susceptible than of resistant lines, when compared with the percentage of infected plants in the parent strains. The range of variability of the F_3 lines did not exceed the limits set by either parent. It seems, from these data, that it would be very difficult to obtain lines in F_3 which are as resistant as the more resistant parent of the cross. The number of genetic factors involved in determining resistance to smut infection could not be determined in these two crosses.

In the F_3 lines of crosses differing in percentage of ear smut, a greater number were more resistant to ear smut than the average of the parents in both of the crosses tested. In the high × low smut cross (28 × 33) the F_3 lines, with one exception, did not exceed the limits set by either parent. In the high × medium smut cross (30 × 17) seven of the fifty-six F_3 lines were more highly infected with ear smut than was the higher smut parent, indicating transgressive segregation in this cross.

Reactions of Selfed Lines Isolated in Regular Corn Breeding Nursery when Tested in Smut Inheritance Plot

Fifty-one selfed lines of corn, which had given a low percentage of infected plants in the regular corn breeding nursery, under normal field conditions, were tested for smut resistance in the smut inheritance plot, in order to determine the reliability of the selection of low smut strains in the regular corn breeding nursery, when the corn was grown under ordinary field conditions. Selfed lines of corn which are highly resistant to smut infection are very difficult to obtain at University Farm. The fifty-one selfed lines referred to were practically all of the strains growing in the regular corn breeding nursery which seemed to be highly smut-resistant under normal field conditions. These fifty-one selfed lines were selected from the selfed lines growing in the corn breeding nursery on the basis of the smut notes taken under normal field conditions over a period of years. They showed promise of being highly smut resistant and were consequently tested in the smut inheritance plot in order to be sure of their resistance. The results of this test are given in Table XV.

Of these fifty-one selfed lines of corn, which were practically all the lines resistant to smut infection under normal field conditions, twenty would be classified as low smut strains, twenty-six as medium smut strains, and five as high smut strains when grown in an artificially induced smut epidemic. Culture 32 is especially interesting because it was very resistant to smut infection for two years under normal field conditions but 100 per cent of the plants were infected by smut when grown in a smut epidemic. Of the seven strains with a percentage of ear smut infection of more than 10, five were in the medium smut group and two in the high smut group.

TABLE XV

PERCENTAGE OF SMUT INFECTION OF STRAINS OF CORN IN THE SMUT PLOT WHICH HAD GIVEN A LOW PERCENTAGE OF SMUT INFECTION FOR SEVERAL YEARS IN THE REGULAR CORN BREEDING NURSERY

Variety	1926 Cult. No.	Percentage total smut						Percentage ear smut		
		Smut plot			Regular breeding plots			Smut plot		
		1926	1925	1924	1923	1922	1921	1926	1925	1924
Rustler	29	80.0	82.4	71.0	0.0	10.0	22.0	7.3	13.2	7.9
N. W. Dent	32	100.0	100.0	100.0	2.0	0.0	39.0	40.0	28.1	12.5
Rustler	34	35.0	55.9	12.2	0.0	0.0	14.0	0.0	10.3	0.0
do	35	16.1	19.5	11.7	7.0	3.0	9.0	3.2	3.7	1.3
Minn. No. 13	36	16.3	20.0	2.0	0.0	0.0	...	2.0	7.1	0.0
N. W. Dent	37	5.1	8.5	4.1	0.0	3.0	37.0	0.0	0.0	0.0
Rustler	44	16.7	9.7	13.0	0.0	3.0	4.0	3.3	0.0	0.0
do	45	9.5	4.4	10.2	0.0	0.0	2.0	0.0	2.2	4.1
do	46*	...	38.0	24.6	0.0	6.0	10.0	...	8.0	3.1
Minn. No. 13	43*	...	89.2	26.3	0.0	0.0	4.0	...	6.5	13.2
do	74†	...	...	43.2	2.0	7.0	...	...	...	3.9
Rustler	80†	...	...	48.8	0.0	0.0	8.0	...	...	5.0
Longfellow	85†	...	...	40.0	7.0	3.0	65.0	...	...	22.0
King Phillip	86†	...	...	53.1	0.0	10.0	7.9	...	...	12.5
		(Smut plot)		(Regular breeding plots)						
Minn. No. 13	46	3.3	0.0	0.0	0.0	15.0	...	0.0	0.0	...
Rustler	47	7.9	8.9	0.0	0.0	0.0	14.0	0.0	1.8	...
do	48	2.3	9.3	3.0	0.0	0.0	7.0	0.0	2.3	...
Minn. No. 13	48*	...	29.0	0.0	3.0	0.0	...	...	5.3	...
do	49*	...	19.6	0.0	3.0	0.0	...	...	3.9	...
do	50*	...	25.5	0.0	0.0	0.0	...	...	0.0	...
do	51*	...	18.7	3.0	3.0	0.0	2.0	...	0.0	...
do	52*	...	47.8	0.0	0.0	0.0	4.0	...	8.8	...
Rustler	54*	...	33.3	0.0	0.0	3.0	4.0	...	22.2	...
King Phillip	56*	...	40.0	0.0	3.0	6.0	11.0	...	13.3	...
do	57*	...	39.1	0.0	3.0	3.0	4.0	...	8.8	...
Minn. No. 13	49	12.9	3.0	0.0	0.0	3.0	...	0.0	...	...
do	50	15.8	3.0	0.0	0.0	3.0	...	0.0	...	...
do	51	9.3	3.0	0.0	0.0	3.0	...	0.0	...	...
do	52	22.2	3.0	0.0	10.0	12.0	...	3.7	...	...
do	53	0.0	0.0	0.0	0.0	37.0	...	0.0	...	...
do	54	19.5	3.0	0.0	3.0	0.0	...	14.6	...	...
do	55	26.8	3.0	0.0	0.0	0.0	...	0.0	...	...
Rustler	56	5.6	0.0	5.0	6.0	...	...	0.0	...	...
do	57	34.7	0.0	0.0	5.0	...	...	12.2	...	...
do	58	30.9	0.0	0.0	8.0	...	...	0.0	...	...
do	59	46.0	0.0	12.0	2.0	...	...	2.7	...	...
do	60	10.8	5.0	3.0	5.0	0.0	4.0	0.0	...	...
do	61	32.1	3.0	4.0	3.0	3.0	18.0	7.2	...	...
Longfellow	62	13.2	8.0	0.0	7.0	1.0	...	5.3	...	...
Silver King	63	9.7	0.0	7.0	0.0	...	...	0.0	...	...
do	64	24.5	0.0	8.0	6.0	...	...	0.0	...	...
do	65	47.9	3.0	...	2.0	6.0	...	0.0	...	...
do	66	28.3	2.0	...	2.0	3.0	...	1.9	...	...
do	67	8.9	0.0	3.0	5.0	5.0	...	4.4	...	...
do	68	19.1	0.0	0.0	3.0	7.0	...	2.1	...	...
do	69	51.4	0.0	10.0	2.0	7.0	...	0.0	...	...
do	70	87.8	0.0	0.0	5.0	8.0	...	7.3	...	...
do	71	37.8	0.0	3.0	3.0	14.0	...	6.7	...	...
do	72	2.3	3.0	0.0	37.0	0.0	...	0.0	...	...
do	73	15.8	0.0	17.0	9.0	3.0	...	2.6	...	...
do	74	6.1	0.0	5.0	0.0	2.0	...	0.0	...	...

* 1925 culture numbers. † 1924 culture numbers.

Thirty-four selfed lines of corn which were being used as parent lines in making single and double crosses in the corn breeding nursery, were tested in the smut inheritance plot in 1926 in order more definitely to determine their reaction to smut infection. These strains were not selected necessarily because of their resistance to smut infection under non-epidemic conditions. They were considered to be the best available parent lines for single and double crosses, as judged by field observation and from notes, one of which was "percentage of smut infection." Some of these strains were known to be quite susceptible to smut infection but were selected because they were the best available, as judged by a large number of factors. The reaction of the thirty-four strains in an artificially induced smut epidemic is given in Table XVI.

TABLE XVI

SELFED LINES OF CORN, USED AS PARENT STRAINS IN SINGLE AND DOUBLE CROSSES IN THE REGULAR PLANT BREEDING NURSERY, TESTED FOR SMUT REACTION IN THE SMUT INHERITANCE PLOT IN 1926

Variety	Plant breeding 1926 Strain No.	Percentage smut	
		Total	Ear
Silver King	2	78.8 ± 6.1	12.7
do	6	52.4 ± 5.8	0.0
do	8	26.1 ± 5.5	0.0
do	1	67.4 ± 7.2	0.0
do	3	26.3 ± 5.5	10.5
do	4	34.0 ± 6.7	10.6
do	5	2.3 ± 0.9	0.0
do	7	25.0 ± 5.2	0.0
do	9	66.0 ± 7.1	1.9
do	10	47.9 ± 5.9	6.3
Minn. No. 13	11	12.7 ± 2.3	3.6
do	12	72.0 ± 5.6	4.0
do	13	15.4 ± 2.8	0.0
do	14	39.6 ± 7.8	20.8
Rustler	15	10.5 ± 1.9	0.0
do	16	75.6 ± 5.9	36.6
do	17	5.9 ± 2.4	0.0
do	18	64.6 ± 6.9	12.5
do	19	38.7 ± 7.7	2.3
do	20	11.1 ± 2.0	0.0
N. W. Dent	21	50.0 ± 6.2	9.4
do	22	55.8 ± 6.1	16.3
do	23	3.7 ± 1.5	0.0
do	24	34.7 ± 6.9	8.2
Longfellow	27	50.0 ± 6.2	26.1
do	26	25.5 ± 5.3	21.3
do	25	73.6 ± 5.7	5.9
do	28	71.5 ± 5.6	9.5
King Phillip	29	78.3 ± 6.1	10.8
do	30	52.9 ± 5.8	9.4
Squaw	31	34.7 ± 6.2	16.3
do	32	34.2 ± 6.8	2.4
Dakota	33	35.1 ± 6.9	10.5
Red Flint	34	41.1 ± 5.1	28.6

Seven of these thirty-four strains would be classified as low smut strains, fifteen as medium smut strains, and twelve as high smut strains, in a smut epidemic, following the method of classification previously described.

It may be concluded from the data in Tables XV and XVI that selection of low smut strains of corn under normal field conditions is an aid in obtaining smut-resistant strains but that these strains should be tested in a smut epidemic in order to be sure of their resistance.

Combination of Eight Low Smut Strains

In 1925 a combination was made of eight strains of corn which seemed to be resistant to smut infection in a smut epidemic, in order to determine whether the factors for smut resistance were the same in all the eight strains and whether anything but low smut lines could be obtained in F_2 and later generations of such a combination.

It has been found, since this combination was made, that one of the eight parent strains was a medium smut strain while the other seven were low smut strains. In Table XVII are given the smut reactions of the F_1 combinations on each of the female parent lines as compared with those of the female parent lines grown the same year.

TABLE XVII

SMUT REACTIONS OF THE F_1 GENERATION OF A COMBINATION OF EIGHT LOW SMUT SELFED LINES GROWN IN 1926

Variety of ♀ parent	Cult. No.	Percentage total smut in		Percentage ear smut in	
		F_1 combination	♀ parent 1926	F_1 combination	♀ parent 1926
Rustler	165	22.6 ± 4.7	0.0	6.5	0.0
do	166	10.6 ± 1.9	35.0 ± 6.9	2.1	0.0
do	167	7.3 ± 3.0	16.1 ± 2.9	0.0	3.2
Minn. No. 13	168	18.0 ± 3.2	16.3 ± 2.9	6.0	2.0
N. W. Dent	169	5.4 ± 2.2	5.1 ± 2.1	1.6	0.0
Longfellow	170	7.3 ± 3.0	9.5 ± 3.9	0.0	0.0
Rustler	171	35.6 ± 7.0	16.7 ± 3.0	5.1	3.3
do	172	14.3 ± 2.6	9.5 ± 3.9	3.6	0.0
Average		14.9 ± 2.7	12.4 ± 2.2	2.9	1.1

None of the F_1 combinations were more susceptible to smut infection than were any of the strains used in making the recombination, when both were tested the same year. The average percentage of total smut infection (obtained by dividing the total number of plants infected by the total number of plants in the eight cultures) was not significantly different from the average percentage infection of the parent cultures. The average percentage of ear smut infection in the F_1 combination was only slightly higher than in the average of the parents. Apparently, the F_1 generation of a combination of low smut strains can be expected to be as resistant to smut infections as the average of the parents; as would be supposed from results obtained from F_1 crosses referred to in the discussion of the reactions of other F_1 crosses.

Location of Smut Boils on the Plants of a Strain

As has already been pointed out (25, 32, 36), the location of smut boils on the plants of a strain of corn is often characteristic of that strain. Further evidence of this was found in the selfed strains grown during the last three years. Thus, culture 74, grown in 1924, showed a peculiar location of smut boils on the necks of the plants (see Fig. 4). In this strain fourteen of the twenty plants infected had smut boils on the necks, that is, just below the tassel.

A brachytic strain, culture 8, grown in 1926, had leaf infections on twenty of the thirty infected plants in the culture. On the other hand, a Rustler strain, culture 38, had 33.4, 42.3, and 41.7 per cent of the plants infected in 1924, 1925, and 1926, respectively, but only one leaf infection during the three years. A Longfellow strain, culture 40, had 52.4, 77.7, and 69.0 per cent of the plants smutted during the same three years and but two leaf infections. On the other hand, the Silver King and Rustler cultures, 70 and 92, grown in 1926, had leaf infections on 67.0 and 72.0 per cent, respectively, of the infected plants.

Ear smut was a strain characteristic in the Longfellow culture 40, in which the percentage of total smut infection in 1924, 1925, and 1926 was 52.4, 77.7, and 69.0 per cent, respectively. Of the infected plants during this three-year period, 76.0, 59.0, and 91.0 per cent, respectively, showed ear smut infections. A strain of King Phillip, culture 39, had 100.0 and 78.0 per cent of the total plants infected by smut in 1925 and 1926, respectively. All infected plants in 1925 had smut infections on the ears. In 1926, 78.0 per cent of the infected plants had ear smut infections.

In 1925 two strains showed an extremely high percentage of tassel infection, and the stamens of one or more branches of the tassel became infected by smut. A Longfellow strain, culture 43, had 15.0, 89.1, and 9.5 per cent of its plants infected by smut in 1924, 1925, and 1926, respectively. The sugary-tunicate strain, culture 12, had 11.9, 95.4, and 12.2 per cent of its plants infected by smut during the same three years. The extremely high percentages of smut in 1925 in these two cultures were due largely to stamen infections. Eighty per cent of the infections of culture 43 and 90.0 per cent of the infections of culture 12 were attributable to tassel (stamen) infection alone in 1925.

A tassel seed strain, culture 10, grown in 1926, contained twenty-four ts_1 ts_1 plants, on which nineteen of the tassel ears were destroyed by smut. When these tassel ears were enclosed in a paper bag, in order to pollinate them artificially, they were practically always destroyed by smut and no seed could be obtained.

In the yellow endosperm strain, culture 5, infections at the base of the plants predominated. This strain showed smut boils on 56.6, 96.2, and 70.2 per cent of all the plants grown in 1924, 1925, and 1926, respectively. During these three years 70.0, 79.0, and 85.0 per cent, respectively, of the infected plants had smut boils at or near the base of the plants. Figure 5 shows base and lower ear bud infections on culture 6.

Infection of the rudimentary ear buds near the base of the plants or at the nodes between the ground and the developed ear (usually nearer the ground than the ear, are very common. This is particularly true late in the season when the plants have practically attained their full development. These shoots, or buds, containing meristematic tissue and being so located that the inoculum may fall or be washed over them, are very often infected by smut. The ear buds are also in a meristematic condition at a time when the temperature is usually high. This favors infection. Some strains are far more susceptible to infection at these locations than are others. For example, the Minnesota No. 13, culture 30, had smut boils on 74.4, 97.4, and 52.2 per cent of the plants in 1924, 1925, and 1926, respectively. Infections were found on the rudimentary ear buds of 50.0, 73.0, and 46.0 per cent of the infected plants during these three years. In the Rustler, culture 34, which showed 12.2, 55.9, and 35.0 per cent of total infection for the three years, 83.0, 68.0, and 90.0 per cent of the infected plants had ear bud infections. In the Northwestern Dent, culture 32, which had all plants infected by smut in 1924 and 1925, 88.0 and 78.0 per cent, respectively, of the infected plants had ear bud infections.

That the percentage of total number of plants infected, in a given strain, is not always an accurate index of its resistance or susceptibility, is shown by the fact that in the four strains having smut boils on more than 70.0 per cent of the plants in 1924, 1925, and 1926, 211 of the 430 smutted plants had more than one infection. In the four low smut strains with an average of less than 12 per cent of smutted plants, grown the same three years under the same conditions, only one of the forty infected plants had more than one smut boil. On plants of susceptible strains smut boils are usually larger than on infected plants of resistant strains. In the cultures mentioned above, 38.2 per cent of the infected plants in the high smut strains produced "large" smut boils and, of the remaining infected plants, 41.2 per cent had "medium" smut boils. Only 17.5 per cent of the infected plants in the low smut strains had large smut boils and of the remaining infected plants 25.0 per cent had medium smut boils.

A Co-operative Study of Smut Reaction Conducted by the Universities of Minnesota and West Virginia

During the seasons of 1925 and 1926, seed of ten selfed lines of corn of known smut reaction, from both Minnesota and West Virginia, was exchanged and seed of the same ears from these twenty selfed lines was planted at both experiment stations and the resulting plants were grown in a smut epidemic. It was expected that this experiment would give some evidence on the reaction of selfed lines of corn to smut infection under different environmental conditions as well as to probably different physiologic forms of corn smut.

In Table XVIII is given the percentage of total and of ear smut infections of these twenty selfed lines when grown at University Farm, St. Paul, Minnesota, and Morgantown, West Virginia, in 1925 and 1926.

TABLE XVIII

MINNESOTA AND WEST VIRGINIA SELFED LINES OF CORN GROWN IN BOTH STATES IN 1925 AND 1926

1926 Minn. Cult. No.	Description	Percentage total smut				Percentage ear smut			
		1925		1926		1925		1926	
		Minn.	W. Va.	Minn.	W. Va.	Minn.	W. Va.	Minn.	W. Va.
		MINNESOTA Selfed Lines							
28	High smut	100.0	62.5	89.2	74.7	26.0	28.2	15.2	25.3
29	do	82.4	26.1	80.0	29.1	13.2	7.2	7.3	0.0
31	Medium smut	47.7	13.6	48.2	8.6	3.1	0.0	7.4	5.2
33	Low smut	3.5	5.9	0.0	6.5	0.0	0.0	0.0	3.0
34	Medium smut	55.9	1.5	35.0	9.7	10.3	0.0	0.0	1.0
35	Low smut	19.5	18.3	16.1	24.4	3.7	1.4	3.2	15.1
36	do	20.0	15.9	16.3	1.6	7.1	0.0	2.0	0.0
37	do	8.5	2.9	5.1	1.0	0.0	0.0	0.0	1.0
39	High smut	...	...	78.0	85.7	...	...	63.4	78.6
41	do	...	...	79.5	9.8	...	...	8.8	2.0
30	do	97.4	18.3	...	...	26.2	1.7	...	...
32	do	100.0	66.7*	...	...	28.1	0.0*	...	...
		WEST VIRGINIA Selfed Lines							
18	Susceptible	98.0	61.5	...	33.3*	2.1	25.6	...	33.3*
19	Resistant	45.5	6.1	39.5	7.7	1.8	0.0	0.0	2.5
20	Susceptible	65.8	25.4	59.6	55.4	11.8	1.4	21.3	8.1
21	do	45.5	18.2†	...	...	13.6	0.0†	...	...
22	Resistant	58.0	10.3	15.9	2.2	16.0	0.0	0.0	2.2
23	do	23.4	1.5	25.0	2.0	0.0	0.0	0.0	0.0
24	do	9.0	0.0	3.9	1.1	0.0	0.0	0.0	1.0
25	Susceptible	92.0	66.7	100.0‡	53.8	34.0	4.4‡	25.0	23.1
26	Resistant	6.6	2.0	0.0*	0.0*	1.1	0.0*	0.0	0.0*
27	Susceptible	79.2	23.4	60.4	40.5	4.2	0.0	0.0	0.0

* 3 plants. † 11 plants. ‡ 4 plants.

A higher percentage of total smut infection was obtained in Minnesota than in West Virginia in all strains tested for two years. All strains which were smut-resistant in Minnesota were also resistant in West Virginia. Strains classed as medium smut lines in Minnesota were medium or low in West Virginia, and high smut strains in Minnesota were high or medium in West Virginia.

The location of smut boils on the plants of a culture, when grown in the two states the same year, was not the same in all instances. In culture 41, in which 79.5 per cent of the plants were infected in Minnesota and only 9.8 per cent in West Virginia in 1926, 47.0 per cent of the plants grown in Minnesota and only 2.0 per cent of those grown in West Virginia had smut boils at the base of the plants. In culture 30, in which the difference in percentage of total plants infected was 79.1 per cent in these two states in 1925, 88.0 per cent of the plants grown in Minnesota and only 7.0 per cent in West Virginia had infections on the main stalk and shoots. However, in this culture, 3.0 per cent of the plants grown in Minnesota and 10.0 per cent of those grown in West Virginia had basal infections.

In culture 18 the percentage of total smut infection was appreciably higher in Minnesota than in West Virginia, while the reverse was true of ear smut. In culture 27, smut infections at the base of the plants predominated in West Virginia in both years of the test, while infections on the main stalk predominated in Minnesota. The same condition was true in culture 32, grown in 1925.

A considerably higher percentage of the West Virginia selfed strains grown in Minnesota in 1926 had smut infections on the tassels than those grown in West Virginia the same year. The reverse was true in 1926. The corn was sprayed with a water suspension of smut conidia during its development in Minnesota, but not in West Virginia. This might account for the higher percentage of tassel smut in Minnesota in 1926. The Minnesota selfed lines had a higher percentage of tassel infection in Minnesota than in West Virginia in 1925. In 1926 some of these strains had an appreciably higher percentage of tassel infections in West Virginia, while in other strains the highest percentage of tassel infection was found in Minnesota.

In general, it may be concluded either that conditions for infection were slightly more favorable in Minnesota than in West Virginia or that the physiologic forms of smut encountered were more virulent in Minnesota than in West Virginia. Some selfed lines, when grown in both states from seed of the same ears, were attacked by smut to very nearly the same extent, while others were slightly more susceptible to infection in one state than in the other. The location of smut boils on the plants was not always the same in the two states in the same year. Smut infections on one location often predominated in one state while most of the infections were located on a different part of the plants in another state. This may have been due to the differential responses of the selfed lines to the probably different physiologic forms of smut. Environmental conditions may have been more favorable for infection in one state than in the other when specific parts of the plants were in a favorable condition for infection.

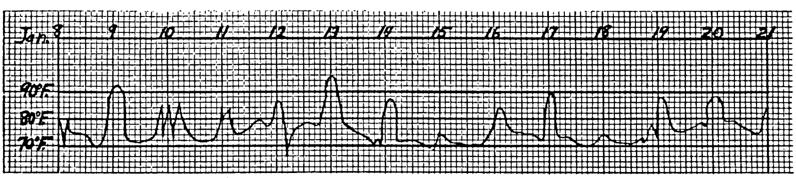
Seedling Infection in the Greenhouse

The same F_3 lines of the Rustler high $\times$ low smut cross (28 $\times$ 33) which were grown in the field in 1925 and 1926, were tested in the greenhouse for the purpose of studying their susceptibility to infection by a single physiologic form of smut. The purpose of this phase of the experiment was to determine the correlation between greenhouse and field notes on smut infection when both were obtained from plants grown from seed of the same ears.

The seeds of these F_3 lines were planted in sand benches in the greenhouse on December 16, 1926. The plants were inoculated hypodermically on January 8, 1927 (see Fig. 6). Notes were taken on January 19 and 21. Table XIX shows the range of temperature in the greenhouse between January 8, when the seedlings were inoculated, and January 21, when the final notes were taken. Figure 7 shows some of the infected seedlings when the notes were taken.

TABLE XIX

RANGE IN TEMPERATURES IN GREENHOUSE BETWEEN TIME OF INOCULATION AND TAKING OF FINAL NOTES



A high temperature seemed to be a far more important factor in determining smut infection than seedling vigor. There seemed to be little correlation between the amount of infection and the apparent vigor of the seedlings. Smut boils appeared first on the seedlings growing in the warmest end of the greenhouse bench, altho the plants were much weaker at this end than at the cooler end of the bench.

In Table XX is presented graphically the range of variability in the percentage of infected seedlings in the F_3 lines. The data from which this graph was made are given in the summary, page 52.

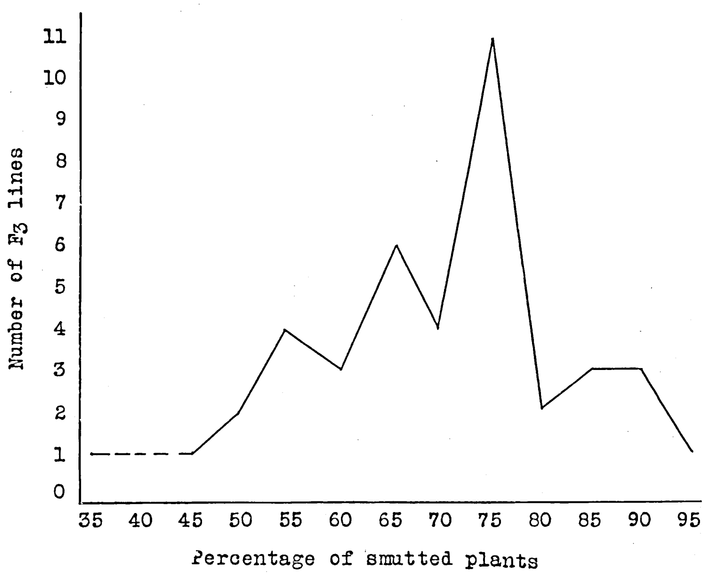
It may be concluded from Table XX that a high percentage of infected corn seedlings may be obtained by hypodermically inoculating with smut sporidia seedlings grown in sand in the greenhouse bench. A mean temperature of between 70° and 80° F. is sufficient for infection and development of smut boils.

There was some variability in the susceptibility of the F_3 lines of this cross to smut infection under the conditions of the experiment;

no lines were found, however, which showed an appreciable amount of resistance. It may be concluded from Table XX that some of the F_3 lines were more resistant than others to infection by this single physiologic form of corn smut, but the differences were not great, considering the small number of plants grown (22 to 48) from each F_3 line.

TABLE XX

SUMMARY OF PERCENTAGES OF SMUT-INFECTED CORN SEEDLINGS IN F_3 LINES OF A RUSTLER HIGH × LOW SMUT CROSS (28 × 33), GROWN IN GREENHOUSE AND ARTIFICIALLY INOCULATED WITH SPORIDIA OF A SINGLE PHYSIOLOGIC FORM OF SMUT



In Table XXI is shown the correlation between the percentage of infection obtained in the greenhouse when the seedlings were artificially inoculated, and the two-year average of infection obtained on the entire plants when grown in the field.

The correlation coefficient of $+0.2854 \pm 0.1434$ indicates a correlation of little significance between the percentages of seedling and field infection. Either conditions determining the amount of smut infection under field conditions were not the same as those in the greenhouse with seedlings hypodermically inoculated with a single physiologic form of smut, or the particular form of smut used was not able to differentiate the F_3 lines grown in the greenhouse in the same way as did the physiologic forms encountered under field conditions. In order to be of practical importance in determining the resistance to smut infection of selfed lines of corn grown in the greenhouse, a physiologic form, or forms, of smut would have to be found which would differentiate the strains in the same way in the seedling stage in the greenhouse as the forms found in the field did under field conditions.

TABLE XXI

Correlation Between Percentage of Seedlings Infected in Greenhouse and of Average Field Infection, Obtained from a Two-Year Test in the Field, of F_3 Lines from a Cross of Rustler High × Low Smut (28 × 33) Lines

Percentage of smutted plants in greenhouse	Average percentage smutted plants in field 1925 and 1926: 18	22	26	30	34	38	42	46	50	54	58	62	66	70	74	Total
36				1												1
40																
44				1												1
48								1								1
52	1	1														2
56						1			1		1					3
60		1							2							3
64			1											1		2
68			2			1		2							1	6
72					1				1		1					3
76		3	2		2	1					1	1				10
80				1		1										2
84						1			1			1				3
88							1	1								2
92												1		1		2
Total	1	5	5	3	3	5	1	4	5		3	3		2	1	41

$r = +0.2857 \pm 0.1434$

Linkage Studies of Smut Reaction

It probably will be impossible to determine the number of genetic factors involved in determining reaction to smut infection until crosses are made and linkage relations determined between the factors determining smut reaction and other factors located in known chromosome linkage groups.

Sufficient material with known genetic factors located in different linkage groups is now available to enable the plant geneticist to determine which of the chromosome linkage groups in corn carry factors for such a quantitative character as smut resistance. It may not be possible, without very intensive study, to establish definitely how many factors are involved in determining reaction to corn smut, but the discovery of which of the ten chromosomes in corn carry factors for smut reaction will be a long step in the solution of this problem.

Corn normally has ten pairs of chromosomes. Eight chromosome linkage groups have been established. In Table XXII is presented a tentative map of the different chromosome linkage groups, showing the relative location of the genes and the distances between them. A number of genes are known to be carried in a particular chromosome, but their location has not been established in relation to more than one other factor in the chromosome. The last group of factors was, therefore, not placed on the chromosome map.

TABLE XXII

EIGHT ESTABLISHED LINKAGE GROUPS IN MAIZE WITH THE LOCI OF MANY OF THE GENETIC FACTORS ON THESE EIGHT CHROMOSOMES

Linkage group	Factors whose location has been determined	Location factors not yet determined	Literature citations
C	I pk C sh wx w_{11} d_3 v_1		4, 12, 14, 24, 34, 35, 66, 67
R	df pg_1 R l_1 g_1 w_2 li	de_2, Pr (?) gm_2, l_2	5, 14, 29, 44, 45, 46, 47, 51, 53, 69
su	su Tu	de, de_1 de_6 ge_1, v_8, wl	14, 22, 23, 39, 53, 70, 72
B	ts_1 v_4 B lg	sk	20, 21, 38
Y	w_5 Y w_6 Pl sm w_1	m_1, m_2	1, 11, 21, 50, 69, 70
P	br P ts_2	ad, f	2, 20, 41, 42
A	A v_3		71
Bn	v_5 gl Bn	pg_3, ra, Y_2, Yp	13, 29, 43

It is, then, with the factors in the above eight known linkage groups that the linkage relations of the factors determining smut reaction may be studied.

In Table XXIII are listed the genetic factors used in studying the linkage relations of the factors for smut reaction.

TABLE XXIII

FACTORS KNOWN TO BE LOCATED IN ONE OF THE EIGHT CHROMOSOME LINKAGE GROUPS AND USED IN STUDYING LINKAGE RELATIONS OF FACTORS FOR SMUT REACTION
(Dominant factors are capitalized.)

Factor	Character
A	Anthocyanin pigment (Emerson, 1918, 1921)
Bn	Brown aleurone (Kvakan, 1924)
br	Brachytic culms (Kempton, 1920)
g_1	Golden plant color (Lindstrom, 1918)
lg	Liguleless leaf (Emerson, 1912)
P	Pericarp color (Emerson, 1911)
Pr	Purple aleurone (East and Hayes, 1911; Emerson, 1918)
Pl	Purple plant color (Emerson, 1921)
R	Aleurone color factor (East and Hayes, 1911; Emerson, 1918, 1921)
sh	Shrunken endosperm (Hutchison, 1921)
sm	Salmon silk (Anderson, 1921)
su	Sugary endosperm (East and Hayes, 1911)
ts_1	Tassel seed (Emerson, 1920)
Tu	Tunicate ear (East and Hayes, 1911; Collins, 1917)
wx	Waxy endosperm (Collins, 1909)
Y_1	Yellow endosperm (East and Hayes, 1911)

Strains of corn carrying these genetic factors were used as parent lines in studying the linkage relations of the factors for smut reaction, and were grown in a smut epidemic to determine their resistance or susceptibility.

The percentage of total and ear smut infection of these strains with factors in known linkage groups is given in Table XXIV.

TABLE XXIV

SPECIAL STRAINS WITH GENETIC FACTORS IN KNOWN LINKAGE GROUPS

Characters and linkage groups	Strain No.	Percentage total smut			Percentage ear smut		
		1924	1925	1926	1924	1925	1926
Waxy endosperm (C), Pr tester (R ?)	1	10.0	22.2	15.8	0.0	4.4	0.0
Shrunken endosperm (C), purple (Y)	2	97.6	100.0	...	87.7	50.0	...
Sugary endosperm (su), tunicate (su)	3	11.9	14.2	12.2	2.4	9.3	4.9
Sugary endosperm (su), R tester (R)	4	30.0	78.2	48.5	16.7	46.9	9.1
Yellow endosperm (Y)	5	56.6	96.2	70.2	3.8	21.6	10.6
Yellow endosperm (Y), liguleless (B)	6	75.0	70.4	75.7	50.0	38.9	21.6
Yellow endosperm (Y), golden (R)	7	7.4	92.4	56.8	5.6	51.3	31.8
Tassel seed—ts_1 (B)	8	...	...	62.5	...	...	2.1
Tassel seed—ts_1 (B)	9	42.6	41.9	...	18.5	6.5	...
Tassel seed—ts_2 (P)	10	37.1	33.1	...	25.7	0.0	...
Brachytic (P)	11	...	...	69.8	...	...	7.0
"A" tester (A)	12	27.3	97.6	71.1	10.9	30.0	20.0
Brown aleurone (Bn)	13	...	66.0	53.6	...	20.8	0.0
Salmon silk (Y)	14	...	57.5	28.0	...	21.3	8.0
Slashed (?)	15	...	...	30.4	...	...	2.2
Rustler glossy (?)	16	...	...	64.0	...	...	8.0

It was from these strains that the parent lines for the linkage study crosses were chosen. Six crosses were made in 1924. The F_1 generations of these crosses were grown the following year. In Table XXV is summarized the percentage of total and ear smut infection of the parent strains and the F_1 crosses of these strains.

In the five crosses in which the F_1 could be compared with the parent strains grown the same year (1925), the F_1 was intermediate in percentage of plants infected in three of the crosses, approached the high smut parent in another, and was as severely infected as the higher smut parent in the one remaining cross. As for ear smut infection, resistance was slightly dominant in four of the F_1 crosses which could be compared with the parents grown the same year, while in the other cross the F_1 most nearly approached the high smut parent.

Four more crosses were made in 1925, involving still other genetic factors, located in linkage groups not dealt with in 1924, as well as factors in other loci of the same chromosomes involved in the 1924 crosses. A summary of the reaction of these crosses is given in Table XXVI.

TABLE XXV

PERCENTAGE TOTAL AND EAR SMUT IN PARENT STRAINS AND F_1 CROSSES MADE FOR LINKAGE STUDY, F_1 GROWN IN 1925

Cross of strains	Description of parent strains	Percentage total smut in parents			Percentage ear smut in parents			Percentage smut F_1 1925	
		1924	1925	1926	1924	1925	1926	Total	Ear
33 ×	Rustler, low smut	4.3	3.5	0.0	0.0	0.0	0.0	45.8±6.8	1.4
2	Purple (Y) shrunken (C), high smut	97.6	100.0*	...	87.7	50.0	...	....	...
33 ×	Rustler, low smut	4.3	3.5	0.0	0.0	0.0	0.0	27.8±5.5	10.8
4	Sugary (su), R tester (R) high smut	30.0	78.2	48.5	16.7	46.9	9.1	....	...
41 ×	Yellow endosperm (Y), high smut	...	95.7	79.5	...	19.5	8.8	79.5±4.3	14.7
33	Rustler, low smut	4.3	3.5	0.0	0.0	0.0	0.0	....	...
33 ×	Rustler, low smut	4.3	3.5	0.0	0.0	0.0	0.0	41.0±6.1	9.6
39	Red pericarp (P), high smut ...	...	100.0	78.0	...	100.0	63.4	....	...
31 ×	Minn. No. 13, medium smut	29.4	47.7	48.2	0.0	3.1	7.4	100.0±2.5	5.9
11	Brachytic (P), high smut	...	...	69.8	...	...	7.0	....	...
36 ×	Minn. No. 13, low smut	2.0	20.0	16.3	0.0	7.1	2.0	70.0±4.8	12.5
6	Liguleless (B), yellow endosperm (Y), high smut ...	77.0	70.4	75.7	50.0	38.9	21.6	....	...

* 2 plants.

TABLE XXVI

PERCENTAGE TOTAL AND EAR SMUT IN PARENT STRAINS AND F_1 CROSSES MADE FOR LINKAGE STUDY, F_1 GROWN IN 1926

Cross of strains	Description of parent strains	Percentage total smut in parents			Percentage ear smut in parents			Percentage smut in F_1 1926	
		1924	1925	1926	1924	1925	1926	Total	Ear
28 ×	Rustler, high smut	83.8	100.0	89.2	5.6	26.0	15.2	91.0±2.5	32.5
3	Sugary (sh), tunicate (su), low smut	11.9	14.2	12.2	2.4	9.3	4.9	....	...
28 ×	Rustler, high smut	83.8	100.0	89.2	5.6	26.0	15.2	49.2±6.1	9.5
1	Waxy (C), Pr tester (R ?), low smut	10.0	22.2	15.8	0.0	4.4	0.0	....	...
28 ×	Rustler, high smut	83.8	100.0	89.2	5.6	26.0	15.2	72.0±5.6	30.7
7	Yellow endosperm (Y), golden (R), medium smut	7.4	92.4	56.8	5.6	51.3	31.8	....	...
9 ×	Ts_1 (B), high smut	42.6	41.9	...	18.5	6.5	...	15.2±2.7	0.0
33	Rustler, low smut	4.3	3.5	0.0	0.0	0.0	0.0	....	...

The F_1 generation of the first cross, that of Rustler high smut × sugary, tunicate, low smut (28 × 3), was as susceptible to total plant infection as the most susceptible parent of the cross when both were grown the same year and more susceptible to ear smut infection. In the other three crosses the F_1 gave an intermediate percentage of total smut when compared with the parent strains. Two of the remaining three crosses were more susceptible to ear smut infection than was either parent, while resistance was dominant in the third.

Five of the six F_1 crosses made for linkage study in 1924 and grown in F_1 in 1925, were grown in F_2 in 1926. While grown in a plot apart from the other selfed lines, and the crosses being tested for smut reaction, they were grown in an artificially induced smut epidemic. Notes were taken on individual plants only once. Tables XXVII to XXXVII give the F_2 progenies of the various crosses involving possible linkages between the factors for smut reaction and known genetic factors in the linkage groups.

In Table XXVII are given the F_2 progenies of the cross low smut, normal endosperm $\times$ high smut, shrunken endosperm (33×2).

TABLE XXVII

F_2 PROGENIES OF CROSS LOW SMUT, NORMAL ENDOSPERM $\times$ HIGH SMUT, SHRUNKEN ENDORPERM (33×2)

Culture No.	Plants from seeds with normal endosperm			Culture No.	Plants from seeds with shrunken endosperm			Percentage plants smutted from	
	Smutted	Not smutted	Total		Smutted	Not smutted	Total	Normal seeds	Shrunken seeds
1, 13, 25	86	205	291	7, 19, 31	29	41	70	29.6	41.4
2, 14, 26	44	173	217	8, 20, 32	11	42	53	20.3	20.8
3, 15, 27	41	128	169	9, 21, 33	9	26	35	24.3	25.7
4, 16, 28	65	110	175	10, 22, 34	9	33	42	37.1	21.4
5, 17, 29	58	152	210	11, 23, 35	17	33	50	27.6	34.0
6, 18, 30	65	142	207	12, 24, 36	29	40	69	31.4	42.0
Total	359	910	1269		104	215	319	28.3	32.6

The difference between the total percentage of infected plants grown from seeds with shrunken and non-shrunken endosperms was only 4.3 per cent. Apparently there was no linkage between the factors for smut reaction and those for shrunken vs. non-shrunken endosperm carried in the "C" linkage group.

Two per cent of the plants grown from non-shrunken seeds had ear smut infections, and 1.9 per cent of those from shrunken endosperm seeds.

In Table XXVIII are summarized the F_2 data on the progenies of the low smut, white aleurone $\times$ high smut, purple aleurone cross (33×2). A total of 984 purple, 105 red, and 890 colorless seeds was obtained in F_2 and these were available for planting. Assuming two color factors, besides the Prpr factors, segregating for color, a 9:7 ratio of colored to colorless would be expected. The obtained segregation fits this expectation very well. The red seeds appear to be much too few for a 3:1 ratio of purple to red. Hayes and Brewbaker (29) suggested that this may be due to a linkage of the Prpr and Rr factors, the latter being carried in the "R" linkage group.

TABLE XXVIII

F_2 PROGENIES OF CROSS LOW SMUT, WHITE ALEURONE × HIGH SMUT, PURPLE ALEURONE (33 × 2)

Culture No.	Plants from seeds with white aleurone			Culture No.	Plants from seeds with red aleurone			Culture No.	Plants from seeds with purple aleurone			Percentage plants smutted from		
	Smutted	Not smutted	Total		Smutted	Not smutted	Total		Smutted	Not smutted	Total	White seeds	Red seeds	Purple seeds
1, 7	60	113	173	25, 31	5	10	15	13, 19	50	123	173	34.7	33.3	28.9
2, 8	25	101	126	26, 32	2	9	11	14, 20	28	105	133	19.8	18.2	21.1
3, 9	21	73	94	27, 33	2	7	9	15, 21	27	74	101	22.3	22.2	26.7
4, 10	30	77	107	28, 34	6	10	16	16, 22	38	56	94	28.0	37.5	40.4
5, 11	23	84	107	29, 35	3	7	10	17, 23	49	94	143	21.5	30.0	34.3
6, 12	36	89	125	30, 36	5	7	12	18, 24	53	86	139	28.8	41.7	38.1
Total	195	537	732		23	50	73		245	538	783	26.6	31.5	31.3

The greatest difference in percentage between smutted plants grown from white, red, and purple aleurone seed was 5.9. It can be concluded from these data that the factors for smut reaction are probably inherited independently of the Prpr factors.

The percentage of plants with ear smut infections was 2.1, 2.1, and 1.8 for the progenies of the white, red, and purple aleurone seeds, respectively.

In Table XXIX are summarized the F_2 data of the progenies of the low smut, starchy endosperm × high smut, sugary endosperm cross (33 × 4).

TABLE XXIX

F_2 Progenies of Cross Low Smut, Starchy Endosperm × High Smut, Sugary Endosperm (33 × 4)

Culture No.	Plants from seeds with starchy endosperm			Culture No.	Plants from seeds with sugary endosperm			Percentage plants smutted from	
	Smutted	Not smutted	Total		Smutted	Not smutted	Total	Starchy seeds	Sugary seeds
37	76	220	296	42	19	50	69	25.7	27.5
38	47	267	314	43	19	56	75	15.0	25.3
39	62	163	225	44	12	41	53	27.6	22.6
40	84	174	258	45	11	51	62	32.6	17.7
41	57	222	279	46	3	40	43	20.4	7.0
Total	326	1046	1372		64	238	302	23.8	21.2

The difference in percentage between the smutted plants grown from starchy and from sugary endosperm seeds was only 2.6. Apparently the factors for smut reaction and for sugary vs. starchy endosperm, located in the "su" linkage group were independently inherited.

The percentage of plants with ear smut infections was 2.6 and 3.3, respectively, for the plants grown from starchy and sugary endosperm seeds.

In Table XXX are summarized the F_2 data of the progenies of the cross (36 × 6) between low smut, ligulate plants × high smut, liguleless plants.

TABLE XXX

F_2 Progenies of Cross Low Smut, Normal Plants × High Smut, Liguleless Plants (36 × 6)

Culture No.	Normal plants			Liguleless plants			Percentage plants smutted	
	Smutted	Not smutted	Total	Smutted	Not smutted	Total	Normal	Liguleless
63, 67, 71	44	182	226	51	49	100	19.5	51.0
64, 68, 72	44	252	296	46	59	105	14.9	43.8
65, 69, 73	22	255	277	52	42	94	7.9	55.3
66, 70, 74	70	202	272	97	14	111	25.7	87.4
75	21	61	82	16	3	19	25.6	84.2
76	83	395	478	84	61	145	17.4	57.9
Total	284	1347	1631	346	228	574	17.4	60.3

In the progenies of each of the six F_1 ears, as well as in the total number of plants, is shown a distinct correlation between susceptibility to smut infection and liguleless plants. The difference in percentage between the normal and liguleless smutted plants was 42.9. Apparently the factor or factors for susceptibility to smut were linked in inheritance with the factors for ligulate vs. liguleless plants, located in the "B" linkage group.

The parent strains of this cross, grown in another plot in 1926, had 21.6 per cent of the liguleless and 2.0 per cent of the ligulate plants infected on the ears. In the F_2, 1.1 per cent of the ligulate plants had ear infections, and 0.9 per cent of the liguleless plants. The percentages were too small to allow any conclusions to be drawn from them, except that there seemed to be no linkage between ear smut and the Lglg factors. These plants matured very late in the season, which probably accounted for the low percentage of ear smut infection.

Smut infections on the base of the plants and on the ear buds predominated in the liguleless parent (see Fig. 5). Smut infections on this part of the plant also predominated in the liguleless plants in the F_2. Smut infections on the ligulate parent strain were not localized, as was also true in the ligulate plant segregates in the F_2.

Susceptibility to smut infection in this cross may have been closely associated with such morphological plant characters as the liguleless condition; it may have been a case of physiological susceptibility determined by genetic factors, or it may have been a combination of the two possibilities, that is, genetic linkage may have been involved in determining susceptibility in the liguleless plants with certain morphological characters of the liguleless plants markedly influencing the expression of the character smut susceptibility. It seems that in this case smut infection on the ear buds was strongly associated with the liguleless condition. Having no ligule, the inoculum could more easily fall upon or be washed over the ear buds in the liguleless plants than in normal plants, whose ear buds have more protection against inoculation. This might account for the susceptibility of the liguleless plants.

In Table XXXI are summarized the F_2 data on the progenies of the cross low smut, green plants $\times$ high smut, purple plants (33×2).

The difference in percentage between the purple and green smutted plants was only 1.7. Apparently the factors for smut reaction were not linked with the Plpl factor pair determining purple and green plants, located in the "Y" linkage group.

TABLE XXXI

F_2 Progenies of Cross Low Smut, Green Plants × High Smut, Purple Plants (33 × 2)

Culture No.	Purple plants: Smutted	Purple plants: Not smutted	Purple plants: Total	Green plants: Smutted	Green plants: Not smutted	Green plants: Total	Percentage plants smutted: Purple	Percentage plants smutted: Green
1, 7, 13, 19, 25, 31	85	188	273	30	58	88	31.1	34.0
2, 8, 14, 20, 26, 32	39	155	194	16	60	76	20.1	21.1
3, 9, 15, 21, 27, 33	33	123	156	17	31	48	21.2	35.4
4, 10, 16, 22, 28, 34	56	112	168	18	31	49	33.3	36.7
5, 11, 17, 23, 29, 35	54	130	184	21	55	76	29.3	27.6
6, 12, 18, 24, 30, 36	73	136	209	21	46	67	34.9	31.3
Total	340	844	1184	123	281	404	28.7	30.4

The purple plant parent of this cross was very susceptible to ear smut infection while the green plant parent was practically immune, under the conditions of the experiment. In F_2, 22 per cent of the purple plants had infected ears and only 9 per cent of the green plants.

In Tables XXXII, XXXIII, and XXXIV are summarized the F_2 data of the progenies of three different crosses between strains differing in smut reaction and color of endosperm.

TABLE XXXII

F_2 Progenies of Low Smut, White Endosperm × High Smut, Yellow Endosperm Cross (33 × 39)

Culture No.	Plants from seed with yellow endosperm: Smutted	Plants from seed with yellow endosperm: Not smutted	Plants from seed with yellow endosperm: Total	Culture No.	Plants from seed with white endosperm: Smutted	Plants from seed with white endosperm: Not smutted	Plants from seed with white endosperm: Total	Percentage plants smutted from: Yellow endosperm seeds	Percentage plants smutted from: White endosperm seeds
47	86	158	244	52	17	57	74	35.2	23.0
48	68	147	215	53	16	49	65	31.6	24.6
49	64	148	212	54	13	48	61	30.2	21.3
50	76	157	233	55	17	49	66	32.6	25.8
51	61	186	247	56	22	42	64	24.7	34.4
Total	355	796	1151		85	245	330	30.8	25.8

TABLE XXXIII

F_2 Progenies of Medium Smut, Yellow Endosperm × High Smut, White Endosperm Cross (31 × 11)

Culture No.	Plants from seed with yellow endosperm: Smutted	Plants from seed with yellow endosperm: Not smutted	Plants from seed with yellow endosperm: Total	Culture No.	Plants from seed with white endosperm: Smutted	Plants from seed with white endosperm: Not smutted	Plants from seed with white endosperm: Total	Percentage plants smutted from: Yellow endosperm seeds	Percentage plants smutted from: White endosperm seeds
57	50	36	86	60	19	24	43	58.1	44.2
58	115	101	216	61	38	31	69	53.2	55.1
59	100	121	221	62	25	18	43	45.2	58.1
Total	265	258	523		82	73	155	50.7	52.9

TABLE XXXIV

F_2 PROGENIES OF CROSS LOW SMUT, YELLOW ENDOSPERM × HIGH SMUT, WHITE ENDOSPERM (36 × 6)

Culture No.	Plants from seed with yellow endosperm			Culture No.	Plants from seed with white endosperm			Percentage plants smutted from	
	Smutted	Not smutted	Total		Smutted	Not smutted	Total	Yellow endosperm seeds	White endosperm seeds
63	76	172	248	67, 71	19	59	78	30.6	24.4
64	64	222	286	68, 72	26	89	115	22.4	22.6
65	53	225	278	69, 73	21	72	93	19.1	22.6
66	123	162	285	70, 74	44	54	98	43.2	44.9
Total	316	781	1097		110	274	384	28.8	28.6

The differences in percentages between the smut-infected plants in the F_2, grown from yellow endosperm seed and from white endosperm seed, in the three crosses summarized in Tables XXXII, XXXIII, and XXXIV, were 5.0, 2.9, and 0.2, respectively. Apparently the factors determining smut reaction and the Yy factor pair for yellow endosperm are not linked.

The percentages of plants having ear smut infections were 9.8 and 7.9 when the plants were grown from yellow and from white endosperm seed, in the first cross (Table XXXII). The percentages with ear smut infections were 2.5 and 1.9, respectively, in the second cross (Table XXXIII); and 0.6 and 1.3, respectively, in the third cross (Table XXXIV).

The factors Yy and Plpl are both located in the "Y" linkage group and are separated by a map distance equivalent to 29.7 per cent crossing over. Since the factors for smut reaction seem to be inherited independently of both the Yy and Plpl factors, which are located a considerable distance apart on the chromosome, the evidence indicates very strongly that the factors for smut reaction are not carried in the "Y" linkage group.

In Table XXXV are summarized the F_2 data of the progenies of the cross low smut, white pericarp × high smut, red pericarp (33 × 39).

TABLE XXXV

F_2 PROGENIES OF CROSS LOW SMUT, WHITE PERICARP × HIGH SMUT, RED PERICARP (33 × 39)

Culture No.	Plants with red pericarp			Plants with white pericarp			Percentage plants smutted with	
	Smutted	Not smutted	Total	Smutted	Not smutted	Total	Red pericarp	White pericarp
47, 52	55	130	185	7	62	69	29.7	10.1
48, 53	29	104	133	4	36	40	21.8	10.0
49, 54	36	113	149	2	47	49	24.2	4.1
50, 55	38	125	163	4	48	52	23.3	7.7
51, 56	23	142	165	8	51	59	13.9	13.6
Total	181	614	795	25	244	269	22.8	9.3

A distinct correlation is evident between smut reaction and pericarp color. More than twice as many of the plants with red pericarp ears were smutted as of plants with white pericarp ears. Apparently the factor, or factors, involved in determining smut reaction in this cross were linked with the Pp factor pair which determined pericarp color. The Pp factors are located in the "P" linkage group.

In this cross 7.2 per cent of the red pericarp plants had ear smut infections and only 2.6 per cent of the white pericarp plants. This is about the same ratio as was found in the case of total smut infection. The red pericarp parent had a very high percentage of ear smut infection (100 per cent in 1925 and 63.4 per cent in 1926) while the white pericarp parent was practically immune.

As no morphological demonstrable plant characters seemed to be associated with the factors for red pericarp color in this cross, that is, morphological characters which might be expected to influence the expression of smut reaction, it seems evident that genetic factors must here be responsible for the linkage between pericarp color and smut reaction.

Selfed strains of corn were found in the smut inheritance plot which all carried the red pericarp color factors (PP) and which varied in percentage of smut infection from 5.0 to 100.0. This suggests that the factors for pericarp color and for smut reaction could be linked in either the coupling or the repulsion phase.

In Table XXXVI are given the F_2 data of the progenies of the cross medium smut, normal plants × high smut, brachytic plants (31 × 11). In this cross twice as many of the brachytic as of the normal plants were infected by smut. Apparently the factor, or factors, determining smut reaction and the Brbr factors, determining the normal vs. brachytic plant characters, were linked in inheritance.

TABLE XXXVI

F_2 PROGENIES OF CROSS MEDIUM SMUT, NORMAL PLANTS × HIGH SMUT, BRACHYTIC PLANTS (31 × 11)

Culture No.	Normal plants			Brachytic plants			Percentage plants smutted	
	Smutted	Not smutted	Total	Smutted	Not smutted	Total	Normal	Brachytic
57, 60	42	57	99	27	3	30	42.4	90.0
58, 61	102	120	222	51	12	63	45.9	81.0
59, 62	76	131	207	49	8	57	36.7	86.0
Total	220	308	528	127	23	150	41.7	84.7

The brachytic parent strain had 7.0 per cent of ear smut in 1926 while the other parent strain had 7.4 per cent. In the F_2, 6.7 per cent of the brachytic plants had ear smut infections and only 1.1 per cent of the normal plants.

Smut infections on the main stalk predominated on the medium smut, normal plant parent. Infections on the leaves and ear buds predominated on the brachytic parent. In the F_2, ear bud infections were a trifle higher (37.3 per cent compared with 25.8 per cent) on the brachytic than on the normal plants. With regard to leaf infection, 32.0 per cent of the brachytic plants were infected and only 1.9 per cent of the normal plants. Of the brachytic plants, 22.6 per cent had main stalf infections in the F_2, while only 5.9 per cent of the normal plants had main stalk infections.

In this brachytic cross, susceptibility may have been determined by genetic factors for smut reaction, or morphological characters of the brachytic plants may have influenced the expression of smut susceptibility. Different parental lines were used in the red pericarp, high smut × white pericarp, low smut cross from those used in the brachytic plants, high smut × normal plants, medium smut cross. In the red pericarp color cross both parents carried the factors for non-brachytic (normal) plants. One of these parent lines was susceptible and one resistant to smut infection. The evidence, therefore, seems quite conclusive that genetic factors were involved in determining smut reaction in these two crosses.

As both the Pp and Brbr factor pairs, located in the "P" linkage group, seem to be linked with a factor or factors determining a specific reaction to smut infection, it may be concluded that factors determining smut reaction are carried in the "P" linkage group.

The parent strains of the cross low smut, white pericarp × high smut, red pericarp (33 × 39), grown in another plot in 1926, showed 0.0 and 78.0 per cent of infected plants in the white and red pericarp parents, respectively. The percentages of infected plants obtained in the F_2 progenies were 9.3 for the white pericarp plants and 22.8 for the red. This indicates that a certain amount of crossing over had occurred between the Pp factors and the factors for smut reaction. The parent strains of the cross medium smut, normal plants × high smut, brachytic plants (31 × 11) had 48.2 and 69.8 per cent of the normal and brachytic plant parent lines, respectively, infected by smut. In the F_2 of this cross, 41.7 per cent of the normal plants were infected and 84.7 per cent of the brachytic plants. It seems from these data that either a closer linkage exists between the factors for smut reaction and the Brbr factor pair than between the factors determining smut reaction and the Pp factor pair, or certain morphological characters of the brachytic plants influenced the amount of smut infection. If genetic factors alone were responsible for smut reaction, the genes would be in this order on the chromosome: pericarp color, smut reaction, brachytic plants; or pericarp color, brachytic plants, smut reaction.

In Table XXXVII are summarized the F_2 data of the progenies of a cross low smut, white aleurone × high smut, brown aleurone (36 × 6).

TABLE XXXVII

F_2 PROGENIES OF CROSS LOW SMUT, WHITE ALEURONE × HIGH SMUT, BROWN ALEURONE (36 × 6)

Culture No.	Plants from seeds with brown aleurone			Culture No.	Plants from seeds with colorless aleurone			Percentage plants smutted from	
	Smutted	Not smutted	Total		Smutted	Not smutted	Total	Brown aleurone seeds	Colorless aleurone seeds
67	15	47	62	71	4	12	16	24.2	25.0
68	16	71	87	72	10	18	28	18.4	35.7
69	20	51	71	73	1	21	22	28.2	4.5
70	32	37	69	74	12	17	29	46.4	41.4
Total	83	206	289		27	68	95	28.7	28.4

The percentages of smutted plants grown from brown aleurone and colorless aleurone seeds were almost identical, indicating that the genes for reaction to smut infection were inherited independently of the Bnbn factors, located in the "Bn" linkage group.

The percentage of plants with ear smut infections in F_2, was 1.7 for plants from brown aleurone seeds and 0.0 for plants from colorless aleurone seeds.

DISCUSSION AND CONCLUSIONS

Selfed lines of corn which seemed to be homozygous for factors determining a particular reaction to smut infection in an artificially induced smut epidemic, were selected as parent strains for making crosses for the purpose of studying the inheritance of smut reaction. It was possible to isolate strains which were practically homozygous for a particular type of smut reaction by selfing for only two or three years. In two of the parent lines used in these crosses, however, it was necessary to self for four or five years before they apparently became homozygous for the factors determining their reaction to smut infection. A uniform percentage of smut-infected plants for three years did not always furnish a reliable guide as to the reaction of these strains in subsequent years. The high correlations between the results obtained from the two series of a uniform replication, as well as the uniform results obtained from year to year, indicate that genetic factors were involved in conditioning a particular type of smut reaction.

Four different F_1 crosses were grown in two different years from seed of the same F_1 ears. Three of these crosses gave practically identical percentages of smut-infected plants in both years. The difference between the reactions of the fourth cross for the two years it was tested was five times its probable error. This difference may have been due to chance variability or it may have been caused by different physiologic forms of *Ustilago zeae* differentiating this cross for the two years of the test.

In eight backcrosses, in which the crosses could be compared with their reciprocals, the differences were all less than three times their probable errors, indicating that the factors were inherited alike in both male and female gametes.

In sixteen F_1 crosses, the reactions to total smut infection of the F_1 were intermediate in twelve crosses, approached the more resistant parent in one cross, and the more susceptible parent in the other three crosses. In six F_2 crosses which could be compared directly with the F_1, the percentage of smutted plants in F_2 was higher in four crosses and lower in two crosses than it was in the F_1. In nineteen backcrosses, twelve approached the more susceptible parent in reaction and seven approached the more resistant parent in percentage of infected plants.

Two crosses were tested in F_3 by growing a large number of F_3 lines from seed of selfed F_2 plants selected at random. In one of these crosses, that of Minnesota No. 13 high $\times$ medium smut (30×17), the percentage of smutted plants was intermediate in the F_1, slightly higher in F_2 than in F_1, the four backcrosses were as susceptible as the more susceptible parent, and in the F_3 there was found a large preponderance of high over medium smut lines. No F_3 lines exceeded the parents in percentage of smutted plants. In the other cross, that of Rustler high $\times$ low smut lines (28×33), in which resistance was slightly dominant in the F_1, the F_2 was slightly more susceptible than the F_1, one backcross was intermediate and the other was as susceptible as the more susceptible parent, the F_3 lines were all medium or high smut lines, no low smut lines being found in F_3. None of the F_3 lines exceeded the reactions of either parent.

In general, in these crosses, there was no dominance of either resistance or susceptibility in F_1; a majority of the F_2 and backcrosses varied in reaction, with a slight tendency for the backcrosses to approach the more susceptible parent; and in F_3 a preponderance of the lines approached, altho they did not exceed, the more susceptible parent in percentage of smut-infected plants.

With regard to ear smut infection, twelve of the sixteen F_1 crosses were intermediate, two approached the resistant parent in reaction, and two approached the more susceptible parent in percentage of ear smut. Five of the six F_2 crosses, which could be compared directly with the F_1, were more susceptible to ear smut infection in the F_2 than they were in the F_1, while the remaining F_2 cross was lower than the F_1. Thirteen of the nineteen backcrosses were intermediate in reaction and the other six were as susceptible as the more susceptible parent. In one of the crosses tested in F_3, in which the F_1 was intermediate, the F_2 was more susceptible than the F_1, and the four backcrosses were intermediate; about half of the F_3 lines were lower and the rest were higher in percentage of ear smut infection than the average of the parents. Seven of the F_3 lines were more susceptible to ear smut than the more susceptible parent. In the other cross tested in F_3, in which resistance to ear smut was slightly dominant in the F_1, the F_2 was more susceptible than the F_1, the backcrosses were almost as susceptible to ear smut infection as the more susceptible parent, forty-one of the F_3 lines were lower and eleven were higher in per cent of ear smut than the average of the parent lines—none of the F_3 lines in this cross exceeded the limits of the parent lines.

In general, the reactions to ear smut infection in these crosses were intermediate in F_1, the F_2 was slightly more susceptible than the F_1 in five of the six crosses, the backcrosses were either intermediate or approached the more susceptible parent in reaction, and slightly more than half of the F_3 lines were more resistant than the average of the parents. The F_1, F_2, and backcrosses were grown the same year, while the F_3 lines were grown in later years. In general, ear smut infection seemed to be inherited in the same way as total smut infection.

A large number of selfed lines of corn which had been grown for several years in the regular corn breeding nursery, were tested in the smut inheritance plot in an artificially induced smut epidemic, in order to determine the reliability of notes taken under normal field conditions, compared with smut epidemic conditions. In general, it was found that notes taken under average field conditions were an aid in obtaining smut-resistant strains, but that these strains should be tested in a smut epidemic in order to be sure of their resistance. If the strains to be so tested have been self-fertilized for three or four years, one year's test in a smut epidemic should give a relatively accurate index as to the actual resistance of the strain. Of the thirteen lines which had been selfed for at least four years and selected as being resistant under normal field conditions, the conclusions based on one year's test in a smut epidemic would have been erroneous in the case of two of these strains, as determined from results obtained by longer tests under epidemic conditions. These two strains probably were not homozygous for the factors determining smut resistance when first tested in the smut inheritance plot.

A combination was made of eight strains which seemed to be smut-resistant, in order to determine whether any lines not smut-resistant would be found in F_2 and later generations of the cross; or if the same genetic factors determined resistance in each of the eight strains combined. The average percentage of total and of ear smut infection was not significantly higher in the F_1 of the combinations than in the average of the parents grown the same year, as might be expected from results obtained in other F_1 crosses. Very nearly the same percentage of smut-infected plants was found in the female parent strains of the Minnesota No. 13, Northwestern Dent, and Longfellow varieties as in the F_1 combinations on these strains. However, considerable variability was found between Rustler female parent strains and the F_1 combinations on these strains. The number of parent strains was too small to allow any conclusions to be drawn from these facts.

A co-operative study was carried on with the department of agronomy of the University of West Virginia in which seed of ten selfed lines of corn of known smut reaction in a smut epidemic, from each state, were exchanged and grown at both Morgantown, West Virginia, and University Farm, St. Paul, in 1925 and 1926. In general, a higher percentage of smut-infected plants was obtained at University Farm than at Morgantown. The same general relationship between the percentage of infected plants in these two localities held for practically all strains involved in the test. Some of the strains were far more severely infected in Minnesota than in West Virginia, indicating either that different physiologic forms of smut were involved or that conditions for infection were more favorable in Minnesota than in West Virginia. The location of the preponderance of smut infections on the plants was not always the same when these strains were grown in both states the same year. As meristematic tissue is necessary for infection by the smut-producing organism, the differences in location of the preponderance of smut boils on the plants of some of the strains may have been due to the fact that environmental conditions necessary for infection were different when specific parts of the plants grown in these two states were most susceptible. Or the physiologic forms of smut encountered in the two states may have differed in their ability to attack specific parts of the corn plants.

The location of smut boils was shown to be a strain characteristic. Strains were found in which infections predominated on leaves, ears, tassels, base, rudimentary ear buds, and necks of the plants. Leaf infections predominated in the brachytic strain, which has a very high leaf area considering the size of the plants. Basal and lower ear bud infections predominated in a liguleless strain, which had less protection against the inoculum washing down behind the leaf and resulting in infection of the ear buds. A very large percentage of the tassel ears in a tassel seed strain were infected by smut. This strain, with its mass of exposed silks at pollination time, is admirably suited for infection at this point. These observations might suggest that the location of smut boils on the plants may be associated with certain morphological characters of the plants.

One of the Minnesota No. 13 strains, on the other hand, had a very large percentage of the infections located on the necks of the plants, while other strains of the same variety had no neck infections. Strains of the variety Rustler were found in which a high percentage of the plants had leaf infections while in other strains of the same variety leaf infections were extremely rare. One strain of Silver King also had a high percentage of leaf infections while in other strains the percentage was small. A selfed strain of Longfellow flint had practically no leaf infections but a high percentage of ear infections. Infections on the ear buds predominated in strains of four different varieties—Minnesota No. 13, Rustler, Northwestern Dent, and a yellow endosperm, liguleless strain—in which no morphologically demonstrable characters were apparent which could be considered as associated with the location of smut boils on the plants. While morphological characters of the plants may be associated with the location of smut boils on specific parts, resistance or susceptibility to smut infection at these locations may also be physiological.

Susceptible selfed strains of corn have a greater tendency toward multiple infections on the plants than have resistant strains. There was also a tendency for smut boils to develop to a greater size on susceptible than on resistant strains.

That the mere "percentage of smut-infected plants" in a given strain, without regard to the location of smut boils on the plants, may lead to erroneous conclusions, was shown in several instances. Three smut-resistant strains have been found which showed an extremely high percentage of infected plants in only one of several years in which they were tested. Upon checking the notes on the location of smut boils on the plants during this year, it was found that in each of the three cases this exceptional increase was due to tassel infection. This occurred but once in each of the three strains referred to, in several years. It would be quite possible to draw erroneous conclusions regarding the degree of resistance of these strains if this factor of the location of smut boils were not taken into consideration.

Seedlings of F_3 lines of a Rustler high $\times$ low smut cross (28×33) were grown in the greenhouse and inoculated hypodermically with smut sporidia of a single physiologic form of *Ustilago zeae*. It was found possible to obtain a high percentage of smut infections on seedlings grown in sand in a greenhouse bench with a mean temperature of between 70° and 80° F. Some variation was found in the amount of infection in these F_3 lines, but no lines with an appreciable degree of resistance were found. No significant correlation, as measured by the correlation coefficient, was found between seedling infection by a single physiologic form of smut, in the greenhouse, and plant infections under field conditions. Either conditions for seedling infection were different from those for plant infections in the field, or the physiologic form of smut used was not able to differentiate these F_3 lines in the same way as were the forms of smut encountered under field conditions.

Crosses were made between selfed strains of corn which differed in smut reaction as well as in genetic factors known to be located in one of the chromosome linkage groups. The linkage relations between the factors determining smut reaction and these other factors were then studied in the F_2 generation.

The factors determining smut infection were found to be inherited independently of the factors for shrunken vs. normal endosperm located in the "C" linkage group, the Prpr aleurone color factors which may be located in the "R" linkage group, the factors for sugary vs. starchy endosperm in the "su" linkage group, the factors for purple vs. green plant color, and the factors for yellow vs. colorless endosperm, both of which are in the "Y" linkage group, and the factors for brown vs. colorless aleurone located in the "Bn" linkage group.

The factors for smut reaction were found to be linked in inheritance with the factors for liguleless vs. ligulate plants located in the "B" linkage group and the factors for brachytic vs. normal plants and red vs. colorless pericarp, both of which are located in the "P" linkage group.

Because of unusual weather conditions in 1926 and the lateness of maturity of these F_2 crosses, the percentage of ear smut was low. However, in the crosses in which the parent strains differed markedly in percentage of ear smut infection, the F_2 segregates showed about the same linkage relations with regard to ear smut as they did with regard to total plant infection.

In the cross of the high smut, liguleless line $\times$ the low smut, normal line (36×6), infections on the base and lower ear buds predominated in the liguleless parent strain. The same condition was true of the liguleless plant segregates in the F_2. In the cross of the high smut, red pericarp line $\times$ the low smut, colorless pericarp line (33×39) there was no particular localization of smut boils on the red pericarp parent. There was a slight preponderance of infections on the ear buds and ears of the red pericarp plant segregates in the F_2. In the cross of the high smut, brachytic line $\times$ the low smut, normal line (31×11), infections on leaves, tassels, and ear buds predominated on the brachytic parent while main stalk infections predominated on the normal parent. In the F_2, infections on the ear buds, leaves, and main stalk predominated on the brachytic plant segregates, while infections on the ear buds, neck, and main stalk predominated on the normal plant segregates.

In the above three crosses there seemed to be some association between the location of smut boils on the plants of the parent strains and on the F_2 segregates of the crosses. There were, however, several instances in which a large percentage of smut boils on a specific location on the plants of one parent was associated with the opposite genotypic type of plants in the F_2. Because of conditions under which the parent strains and the F_2 crosses were grown in 1926, however, it would be difficult to draw definite conclusions regarding the extent to which location of smut boils on the plants of a parent strain was associated with the particular type of plants in the F_2.

SUMMARY

1. Inheritance of reaction to smut *(Ustilago zeae)* was studied in selfed lines of corn and crosses between these selfed lines.

2. Selfed lines of corn which were apparently homozygous for a particular type of smut reaction were isolated after only a few years of selfing.

3. Factors determining smut reaction were transmitted alike in both male and female gametes.

4. Crosses between selfed lines differing in smut reaction were, in general, intermediate in reaction. There were several cases of apparent dominance of susceptibility, however. A slight tendency to approach the more susceptible parent was found in the F_3 and backcrosses. Reaction to ear smut infection was inherited in the same manner as reaction to total smut infection.

5. Selection of selfed lines of corn resistant to smut under average field conditions was found to be an aid in obtaining such resistant strains, but they should be tested in a smut epidemic in order to be sure of their resistance.

6. F_1 combinations of eight smut-resistant strains were as resistant as the average of the parent strains.

7. The relationship between the percentage of smut-infected plants was quite constant for the same strains grown both in Minnesota and in West Virginia the same two years. The location of smut boils on the plants differed somewhat when grown in the two states. This may have been due to different physiologic forms of smut or to the fact that conditions for infection at different stages of the growth of the plants were not the same.

8. Location of smut boils on the plants was found to be a strain characteristic. Morphological characters of the plants may have been associated with the location of smut boils on the plants or physiologic susceptibility may have determined their location.

9. Seedling infection was studied in the greenhouse. No appreciable correlation was found between the amount of seedling infection with a single physiologic form of smut and plant infection under field conditions.

10. Linkage relations were studied between the factors determining smut reaction and one or more known genetic factors in seven of the chromosome linkage groups. The factors for smut reaction were found to be inherited independently of the Shsh, Prpr, Susu, Plpl, Yy, and Bnbn factor pairs, which are located in five different linkage groups, and to be linked with the factor pairs Lglg, Brbr, and Pp, which are located in two different linkage groups.

Summary of Reactions of F_3 Lines of Rustler High × Low Smut Cross (28 × 33)

The seedlings were grown in the greenhouse and artificially inoculated with sporidia of a single physiologic form of smut.

1926 Culture No.	No. of plants	Per cent smutted	1926 Culture No.	No. of plants	Per cent smutted
109	32	53.1	138	34	61.8
110	41	36.6	139	33	87.9
111	38	44.7	140	34	76.5
112	42	85.7	141	31	77.4
113	35	60.0	142	34	70.6
114	43	55.8	143	46	76.1
115	39	66.7	144	35	74.3
119	41	58.5	145	45	51.1
116	37	48.6	146	40	85.0
117	35	68.6	147	33	78.8
118	33	66.7	148	40	77.5
120	36	66.7	150	41	92.7
121	40	72.5	151	44	88.6
122	35	85.7	153	44	75.0
123	35	57.1	156	44	77.3
126	36	55.6	157	38	65.8
127	30	66.7	158	48	77.1
124	22	68.2	159	44	77.3
128	35	80.0	160	43	74.4
133	31	90.3	162	44	72.7
136	25	64.0			

LITERATURE CITED

1. Anderson, E. G. The inheritance of salmon silk color in maize. New York (Cornell) Agr. Exp. Sta. Memoir 48. 1921.
2. ——— and Emerson, R. A. Pericarp studies in maize. I. The inheritance of pericarp colors. Genetics **8**:466-476. 1923.
3. Arthur, J. C. and Stuart, W. Corn smut. Indiana Agr. Exp. Sta. Ann. Rept. (1899) **12**:84-135. 1900.
4. Bregger, T. Linkages in maize. The C aleurone factor and waxy endosperm. Am. Nat. **52**:57-61. 1918.
5. Brunson, A. M. The inheritance of a lethal pale green seedling character in maize. New York (Cornell) Agr. Exp. Sta. Memoir 72. 1924.
6. Christensen, J. J. and Stakman, E. C. Physiologic specialization and mutation in *Ustilago zeae*. Phytopath. **16**:979-1000. 1926.

7. Coffman, F. A., Tisdale, W. H., and Brandon, J. F. Observations on corn smut at Akron, Colorado. Jour. Am. Soc. Agron. 18:403-410. 1926.
8. Collins, G. N. A new type of Indian corn from China. U. S. Dept. Agr. Bur. Plant Ind. Bull. 161. 1909.
9. ——— Gametic coupling as a cause of correlation. Am. Nat. 46:569-590. 1912.
10. ——— Hybrids of *Zea ramosa* and *Zea tunicata*. Jour. Agr. Res. 9:383-395. 1917.
11. Demerec, M. Inheritance of white seedlings in maize. Genetics 8:561-593. 1923.
12. ——— Genetic relations of five factor pairs for virescent seedlings in maize. New York (Cornell) Agr. Exp. Sta. Memoir 84. 1924.
13. ——— Inheritance of pale green seedlings in maize. Genetics 10:318-344. 1925.
14. ——— Notes on linkages in maize. Am. Nat. 60:172-176. 1926.
15. East, E. M. and Hayes, H. K. Inheritance in maize. Conn. Agr. Exp. Sta. Bull. 167. 1911.
16. ——— and Jones, D. F. Genetic studies on the protein content of maize. Genetics 5:543-610. 1920.
17. Emerson, R. A. Genetic correlation and spurious allelomorphism in maize. Neb. Agr. Exp. Sta. Ann. Rept. 24:58-90. 1911.
18. ——— The inheritance of the ligule and auricle of corn leaves. Neb. Agr. Exp. Sta. Ann. Rept. 25:81-88. 1912.
19. ——— A fifth pair of factors, Aa, for aleurone color in maize and its relation to the Cc and Rr pairs. New York (Cornell) Agr. Exp. Sta. Memoir 16. 1918.
20. ——— Heritable characters of maize. II. Pistillate flowered maize plants. Jour. Heredity 11:65-76. 1920.
21. ——— The genetic relations of plant colors in maize. New York (Cornell) Agr. Exp. Sta. Memoir 39. 1921.
22. Eyster, W. H. The linkage relations between the factors for tunicate ear and starchy-sugary endosperm in maize. Genetics 6:209-240. 1921.
23. ——— The intensity of linkage between the factors for sugary endosperm and for tunicate ears and the relative frequency of their crossing over in microspore and megaspore development. Genetics 7:597-601. 1922.
24. ——— Heritable characters in maize: Polkadot leaves. Jour. Heredity 15:397-400. 1924.
25. Garber, R. J. and Quisenberry, K. S. Breeding corn for resistance to smut *(Ustilago zeae)*. Jour. Am. Soc. Agron. 17:132-139. 1925.
26. Griffee, Fred. Factor relations in barley species crosses. (Abs.) Anat. Rec. 26:384. 1923.
27. ——— Correlated inheritance of botanical characters in barley, and manner of reaction to *Helminthosporium sativum*. Jour. Agr. Res. 30:915-935. 1925.
28. Hayes, H. K. Production of high-protein maize by Mendelian methods. Genetics 7:237-257. 1922.
29. ——— and Brewbaker, H. E. Factors for color of aleurone and endosperm in maize. Jour. Am. Soc. Agron. 18:761-766. 1926.
30. ——— and Garber, R. J. Synthetic production of high protein corn in relation to breeding. Jour. Am. Soc. Agron. 11:309-318. 1919.
31. ——— Breeding crop plants. McGraw-Hill Book Company, New York. 1921.

32. ———, Stakman, E. C., Griffee, Fred, and Christensen, J. J. Reactions of selfed lines of maize to *Ustilago zeae*. Phytopath. 14:268-280. 1924.

33. Hitchcock, A. S., and Norton, J. B. S. Corn smut. Kansas Agr. Exp. Sta. Bull. 62:169-212. 1896.

34. Hutchison, C. B. Heritable characters of maize. VII. Shrunken endosperm. Jour. Heredity 12:76-83. 1921.

35. ——— The linkage of certain aleurone and endosperm factors in maize, and their relation to other linkage groups. New York (Cornell) Agr. Exp. Sta. Memoir 60. 1922.

36. Immer, F. R. and Christensen, J. J. The reaction of selfed lines and crosses of maize to *Ustilago zeae*. Phytopath. 15:699-707. 1925.

37. Jones, D. F. Segregation of susceptibility to parasitism in maize. Am. Jour. Bot. 5:295-300. 1918.

38. ——— Heritable characters of maize. XXIII. Silkless. Jour. Heredity 16:339-341. 1925.

39. ——— and Gallastegui, C. A. Some factor relations in maize with reference to linkage. Am. Nat. 53:239-246. 1919.

40. Kempton, J. H. Heritable characters of maize. III. Brachytic culms. Jour. Heredity 11:111-115. 1920.

41. ——— Linkage between brachytic culms and pericarp and cob color in maize. Jour. Wash. Acad. Sci. 11:13-20. 1921.

42. ——— Linkage between brachyism and adherence. Am. Nat. 56:461-464. 1922.

43. Kvakan, Paul. The inheritance of brown aleurone in maize. New York (Cornell) Agr. Exp. Sta. Memoir 83. 1924.

44. Lindstrom, E. W. Linkages in maize: Aleurone and chlorophyll factors. Am. Nat. 51:225-237. 1917.

45. ——— Chlorophyll inheritance in maize. New York (Cornell) Agr. Exp. Sta. Memoir 13. 1918.

46. ——— Chlorophyll factors of maize: Their distribution on the chromosomes and relation to the problem of inbreeding. Jour. Heredity 11:269-277. 1920.

47. ——— Concerning the inheritance of green and yellow pigments in maize seedlings. Genetics 6:91-110. 1921.

48. ——— Heritable characters in maize. XIII. Endosperm defects. Sweet defective and flint defective. Jour. Heredity 14:127-135. 1923.

49. ——— A genetic linkage between size and color characters in the tomato. Science 60:182-183. 1924.

50. ——— Complementary genes for chlorophyll development in maize and their linkage relations. Genetics 9:305-326. 1924.

51. ——— Genetic factors for yellow pigment in maize and their linkage relations. Genetics 10:442-455. 1925.

52. ——— Heritable correlation of size and color characters in tomatoes. Iowa Agr. Exp. Sta. Res. Bull. 93. 1926.

53. Mangelsdorf, P. C. The genetics and morphology of some endosperm defects in maize. Conn. Agr. Exp. Sta. Bull. 279. 1926.

54. Melchers, L. E. Ecological and physiological notes on corn smut, *Ustilago zeae*. (Abs.) Phytopath. 11:32. 1921.

55. Pammel, L. H., and Stewart, F. C. Prevention of corn oat smut. Iowa Agr. Exp. Sta. Bull. 20:721-726. 1893.

56. Piemeisel, F. J. Factors affecting the parasitism of *Ustilago zeae*. Phytopath. 7:294-307. 1917.

57. Plant Disease Reporter of the Bureau of Plant Industry of the U.S. Dept. of Agr. Dec. 30, 1924.
58. ——— Oct. 1, 1925.
59. ——— Nov. 1, 1926.
60. Potter, Alden A. and Melchers, L. E. Study of the life history and ecological relations of the smut of maize. Jour. Agr. Res. **30**:161-173. 1925.
61. Sartoris, G. B. Studies in the life history and physiology of certain smuts. Am. Jour. Bot. **11**:617-647. 1924.
62. Sax, Karl. The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics **8**:552-560. 1923.
63. ——— The nature of size inheritance. Nat. Acad. Sci. Proc. **10**:224-227. 1924.
64. ——— Quantitative inheritance in Phaseolus. Jour. Agr. Res. **33**:349-354. 1926.
65. Sirks, M. J. The inheritance of seed weight in the garden bean. Genetica **7**:119-169. 1925.
66. Stadler, L. J. Variation in the intensity of linkage in maize. Am. Nat. **59**:366-370. 1925.
67. ——— The variation of crossing over in maize. Genetics **11**:1-37. 1926.
68. Stakman, E. C. and Christensen, J. J. Physiologic specialization of *Ustilago zeae* and *Puccinia sorghi* and their relation to corn improvement (Abs.) Phytopath. **16**:84. 1926.
69. Stroman, G. N. Genetic relations of chlorophyll and anthocyanin seedling characters in maize. Genetics **9**:91-123. 1924.
70. ——— The inheritance of certain chlorophyll characters in maize. Genetics **9**:493-512. 1924.
71. Tisdale, W. H. and Johnston, C. O. A study of smut resistance in corn seedlings grown in the greenhouse. Jour. Agr. Res. **32**:649-668. 1926.
72. Wentz, J. B. Linkage between sweet defective and sugary endosperm in maize. Genetics **10**:395-401. 1925.
73. Wood, T. B. and Stratton, F. J. M. The interpretation of experimental results. Jour. Agr. Sci. **3**:417-440. 1910.

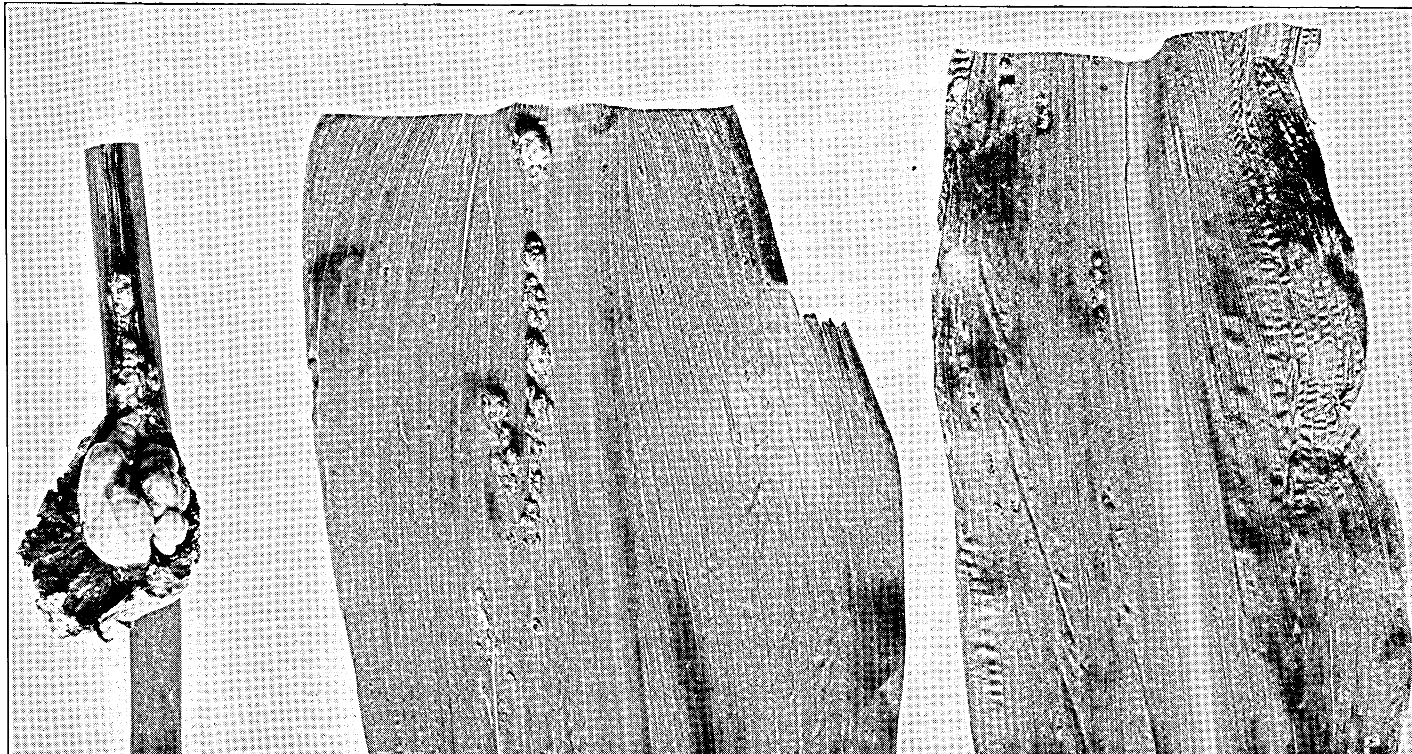


Fig. 1. "Incipient," "Warty," and "Small" Smut Boils

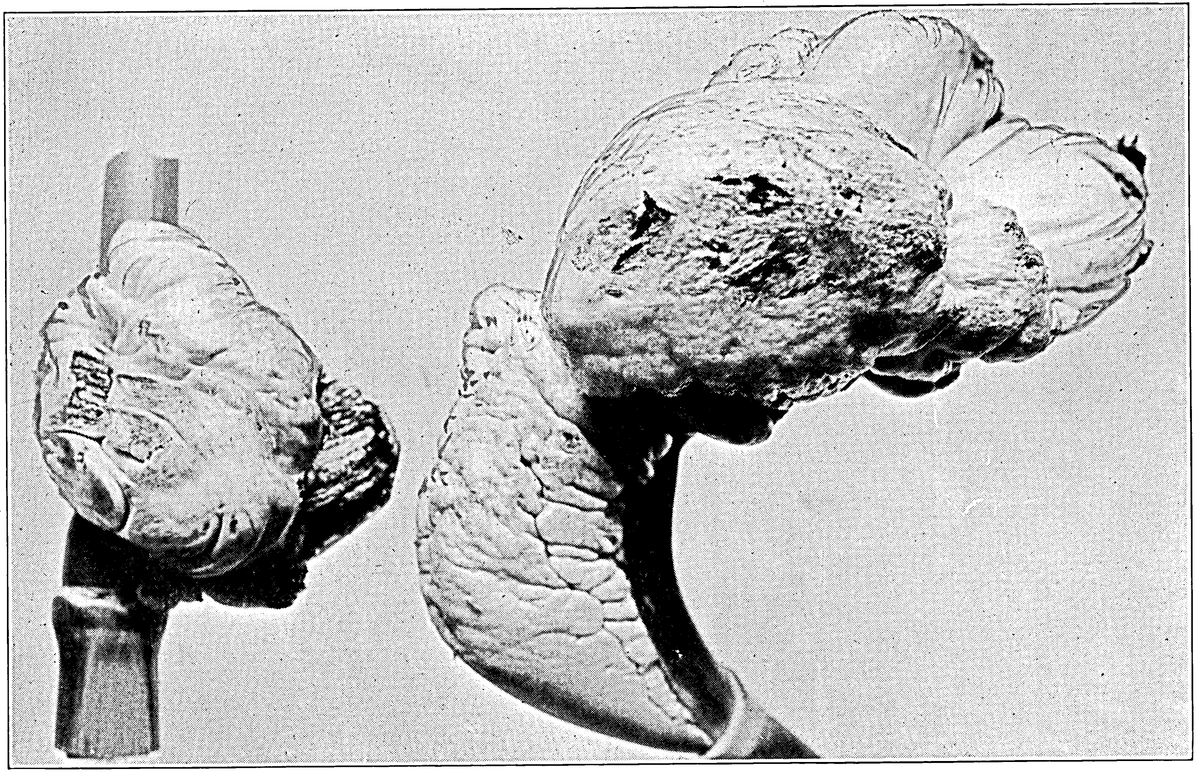


Fig. 2. "Medium" and "Large" Smut Boils, Used as a Basis in Classifying Size of Smut Boils

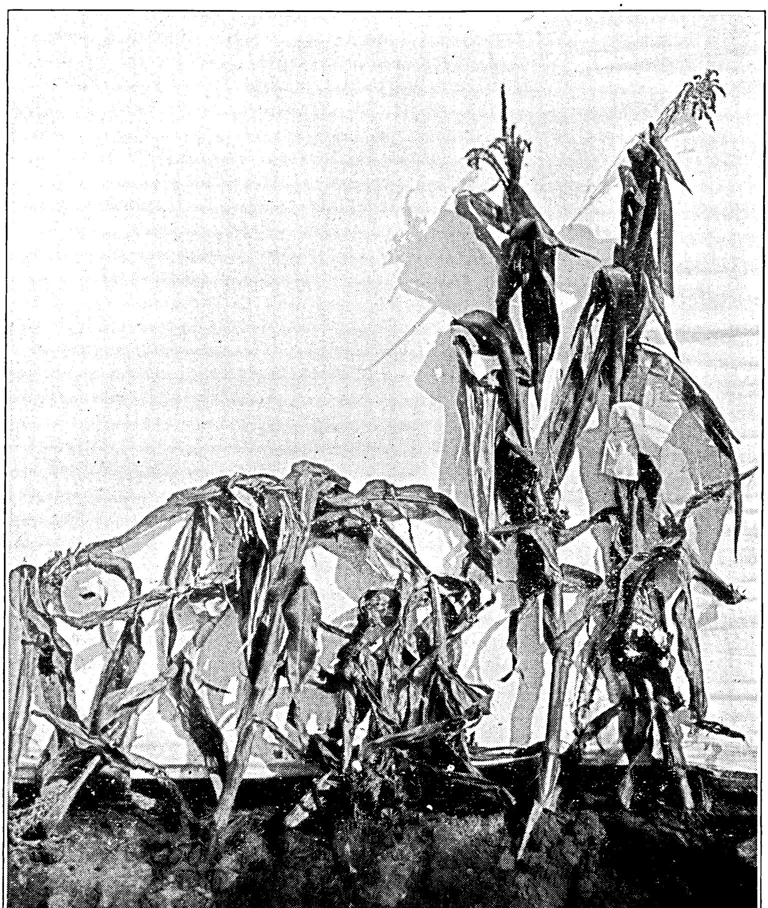


Fig. 3. Rustler High Smut Strain, Culture 28

Fig. 4. Minnesota No. 13 Strain Showing Smut Boils on Necks of Plants as a Characteristic of this Strain

Fig. 5. Base and Lower Ear Bud Infections on a Yellow Endosperm Liguleless Strain, Culture 6

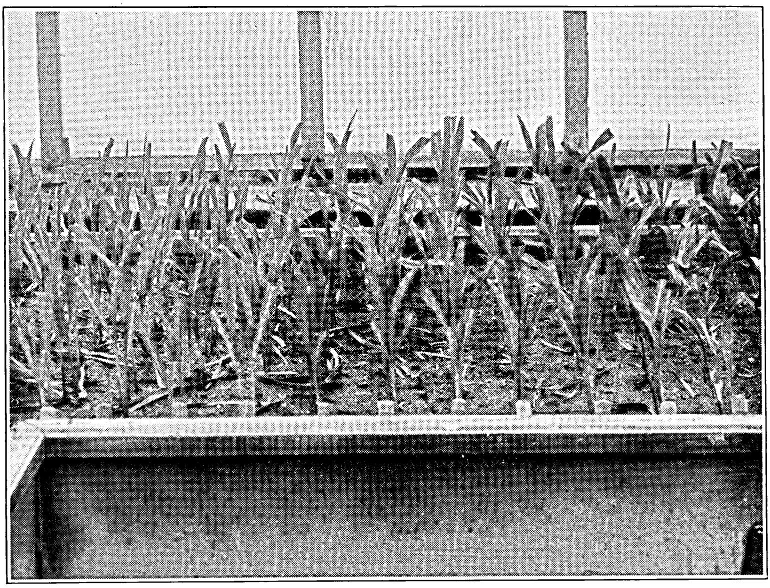


Fig. 6. Corn Seedlings Growing in Sand in the Greenhouse Bench on January 8, 1927, the Day They Were Inoculated, Hypodermically, with Smut Conidia

Fig. 7. Smut Infections on Corn Seedlings Inoculated in the Greenhouse with Smut Conidia, Showing Location and Degree of Smut Infections on Seedlings Photographed Jan. 21, 1927, the day smut infection notes were taken.